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Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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Epidemiology Investigations, Crops Protection Research Branch
Plant Industry Station, Beltsville, Maryland

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CONTENTS

1. Common weed hosts of tobacco ringspot virus in the Lower Rio Grande Valley of Texas
D. M. McLEAN 5

2. Crazy top of corn in Mississippi
JAMES W. BROYLES and C. O. GROGAN 8

3. The effects of stalk rot on corn yields in Illinois
A. L. HOOKER and M. P. BRITTON 9

4. Additional sources of resistance to Puccinia sorghi in the United States
A. L. HOOKER 14

5. The occurrence of Cytospora canker on spruce in the presence of Xiphinema americanum and Criconemoides xenoplax
A. H. EPSTEIN and G. D. GRIFFIN 17

6. Stalk rot of corn in the Egyptian Region
HOSNI A. MOHAMED 18

7. Attempts to eliminate sweetpotato internal cork virus by heat treatments
W. J. MARTIN 19

8. Elimination of root-knot nematodes from infested sweetpotato roots and plants
W. J. MARTIN 21

9. Pathogenicity of the stubby root nematode to onion
JOHN K. HOFF and W. F. MAI 24

10. Interfertility study of Polyporus palustris and other brown rot species occurring on conifers and hardwoods
HAZEL H. McKAY 26

11. Undescribed plant diseases and new hosts recorded in Oregon during 1960-1961
ROBERT S. HALLIWELL and IAIN C. MacSWAN 30

12. Control of postharvest decays of peaches
R. A. CAPPELLINI and A. W. STRETCH 31

13. Preliminary list of pathogenic fungi of foreign forest trees planted in Tokyo University Forest, Hokkaido, Japan
HARUYOSHI SAHO 34

14. Susceptibility of wildfire-resistant tobacco varieties to certain strains of Pseudomonas tabaci and P. angulata
W. D. VALLEAU, et al. 36

15. An iron deficiency of Para grass, Brachiaria mutica, and Guinea grass, Panicum maximum, noted in the western region of Nigeria
WARREN N. STONER 38

16. Watermelon diseases on the Chicago market 1960-1961
N. S. LUEPSCHEN and M. A. SMITH 41

17. Effects of pretreatment of Heterodera schachtii cysts with sugar solutions on emergence of larvae in sugar-beet root diffusate
ARNOLD E. STEELE 43

18. Effect of simulated freeze-cracking on invasion of dry-ice injured stems of Stanley prune trees by naturally disseminated Cytospora inoculum
A. W. HELTON 45

19. Effects of seed inoculation, soil fumigation, and cropping sequences on soybean nodulation in soybean-cyst-nematode-infested soil
JAMES M. EPPS and ALBERT Y. CHAMBERS 48

20. Preliminary report on a Meloidogyne sp. parasite of grass in Florida
E. B. SLEDGE 52

21. Sclerotinia blight of Bahia and Coastal Bermuda grasses
DOUGLAS C. BAIN 55

22. Inoculations with the sooty-bark canker fungus on aspen
THOMAS E. HINDS 57

23. A decline condition in pears induced by pear psylla
R. C. LINDNER, et al. 59

24. Inexpensive temperature tanks
GEORGE FASSULIOTIS 61

25. A laboratory method of obtaining sporophores of Fomes annosus
JOHN S. BOYCE, Jr. 63

26. Fomes annosus infection through pruned branches of slash pine
WESLEY WITCHER and ROBERT E. BEACH 64

27. Covered smut of crabgrass
EDGAR C. TULLIS 64

28. Peanut rust in Virginia
GROVER C. SMART, Jr. 65

29. Peanut rust in North Carolina
J. C. WELLS 65

COMMON WEED HOSTS OF TOBACCO RINGSPOT VIRUS IN THE LOWER RIO GRANDE VALLEY OF TEXAS

D. M. McLean[1]

Tobacco ringspot virus (TRSV) is the most prevalent virus affecting cucurbits in the Lower Rio Grande Valley (3). It also is important in certain other vegetables (2). During 1959-61 studies were conducted in the field and in the glasshouse to detect weed species which serve as TRSV reservoirs. Weed species with ringspot symptoms were indexed to TRSV-sensitive test plants (3). When such test plants indicated the presence of TRSV, seedlings of the respective weed species were inoculated with a known strain of the virus (2), and symptoms were compared with those observed in natural field-infected plants. All inoculations were made manually using triturated juice extract and carborundum (400) applied with a cheesecloth pad.

A list of susceptible species and description of symptoms in plants considered important reservoirs of the ringspot virus in the Lower Rio Grande Valley is given in Table 1. Symptoms in several selected common species are illustrated in Figure 1. TRSV is endemic in certain weeds common in the region and 13 species are listed as new hosts. Several unreported hosts not common to the region are included also.

Table 1. Symptoms produced on plants considered important reservoirs of TRSV in the Lower Rio Grande Valley.

Susceptible species	New hosts	Local symptoms	Systemic symptoms	Abundance
Amaranthus palmeri[a]	x	necrotic specks on leaves	chlorotic specks, ringspotting, mild mottle, recovery - Fig. 1,A	very common
A. retroflexus[a]	x	do.	do.	do.
Gomphrena globosa	x	none	chlorotic stipple, ringspotting, slight rugosity and mottle, recovery	uncommon
Chenopodium murale[a]	x	necrotic specks on leaves	chlorotic specks in young leaves, terminal necrosis, recovery	very common in winter
C. album[a] (5)[b]		do.	do.	do.
C. amaranticolor	x	necrotic spots with reddish borders	chlorotic specks in young leaves, crinkle, recovery	exotic
Helianthus annuus[a] (5)		none	mild ringspotting, faint mottle-fading, recovery - Fig. 1,B	very common
Boerhaavia erecta[a]	x	reddish necrotic lesions, leaf abscission	terminal necrosis, often lethal	do.
B. coccinea[a]	x	none	interveinal chlorosis, vein-banding, chlorotic stipple, wavy leaf margins - Fig. 1,C	do.
Abutilon theophrasti (1)		none	interveinal chlorosis, dark green veins	uncommon
Hibiscus cannabinus	x	none	faint mottle, leaves curled upwards, necrotic spots in young leaves, partial recovery Fig. 1,H	do.

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[1] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, in cooperation with the Texas Agricultural Experiment Station, Weslaco, Texas.

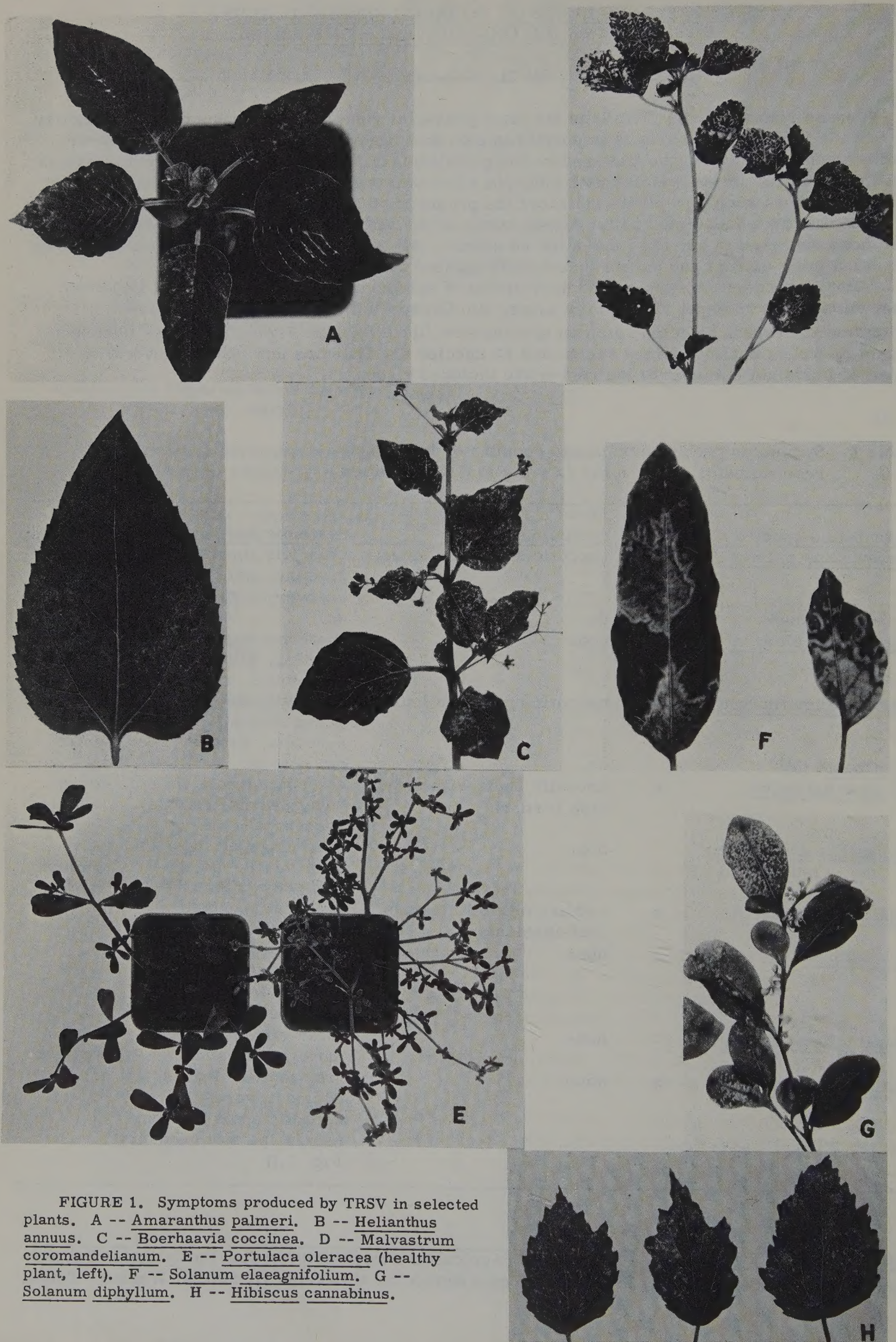

FIGURE 1. Symptoms produced by TRSV in selected plants. A -- *Amaranthus palmeri*. B -- *Helianthus annuus*. C -- *Boerhaavia coccinea*. D -- *Malvastrum coromandelianum*. E -- *Portulaca oleracea* (healthy plant, left). F -- *Solanum elaeagnifolium*. G -- *Solanum diphyllum*. H -- *Hibiscus cannabinus*.

Table 1. (Continued)

Susceptible species	New hosts	Local symptoms	Systemic symptoms	Abundance
Malvastrum coromandelianum[a]	x	none	vein clearing and banding, chlorotic spots, mild mottle - Fig. 1,D	very common
Portulaca oleracea[a]	x	none	mild chlorotic spots, leaves turn reddish purple, pronounced reduction in leaf size - Fig. 1,E	do.
Solanum diphyllum (listed in Standley's, Trees and Shrubs of Mexico)	x	none	chlorotic ringspots and specks, mottling, symptoms persistent - Fig. 1,G	uncommon (ornamental)
S. elaeagnifolium[a] (2)		none	Ringspotting, calico yellows, zig-zag patterns, chlorosis - Fig. 1,F	very common
Physalis floridana	x	none	slight ringspotting after 5 days, recovery followed by interveinal chlorosis, sometimes yellow vein banding	uncommon
Margaranthus solanaceus[a]	x	none	slight vein clearing, chlorotic stipple	common
Datura stramonium (5)		none	ringspotting, chlorotic specks, interveinal chlorosis	uncommon
D. metel (4)		none	do.	do.

[a]Hosts considered as important reservoirs of TRSV in the Lower Rio Grande Valley.
[b]Species previously found to be susceptible by other workers.

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CRAZY TOP OF CORN IN MISSISSIPPI[1]

James W. Broyles and C. O. Grogan[2]

In 1954 a disease similar to that described as crazy top by Ullstrup[3] was found by the senior author affecting corn tassels in a small, low, wet area in Noxubee County, Mississippi. The typical curved witches' broom generally associated with crazy top was present. Histological sections of deformed tissues revealed oospores similar to those described for Sclerospora macrospora (Sacc.) Thirum., Shaw & Naras. (Sclerospora macrospora Sacc.). As far as the authors know, this disease had never been found before on corn in Mississippi though the fungus S. macrospora had been reported on oats and grasses[4].

The disease was not noted again in the State until 1959, when the authors observed a condition in corn at State College, suggested by others as a possible abnormal response to nitrogen. Late in the season many plants were abnormally tall and vigorous and still light green where normal plants were turning brown. Barrenness of infected plants evidently accounted for this extended vegetative period. Oospores similar to those of S. macrospora were again found in deformed tissues, and examples of the entire syndrome of crazy top, as previously described[3], were present. Giantism, an additional effect of the disease, has not been described previously in the literature.

Superficially the oversized plants appeared exceptionally vigorous and healthy except for being light green (Fig. 1). Infected plants exhibited different degrees of giantism, some being over 13 feet tall with relatively large stalks. Many had almost twice the normal number of leaves and internodes. Whereas a normal plant had about 8 internodes above the top ear shoot and 13 below, infected plants often had 15 internodes above the top ear shoot and 21 below. Some plants had six elongated ear shoots but with little or no grain and often no cob. Most ear shoots on infected plants were abnormally long, sometimes 30 inches or more. Instead of the normal 10 leaves and internodes affected shoots had 20 leaves and internodes but no cob. Some ear shoots, however, appeared to proliferate and form several short, deformed, barren shoots at one node.

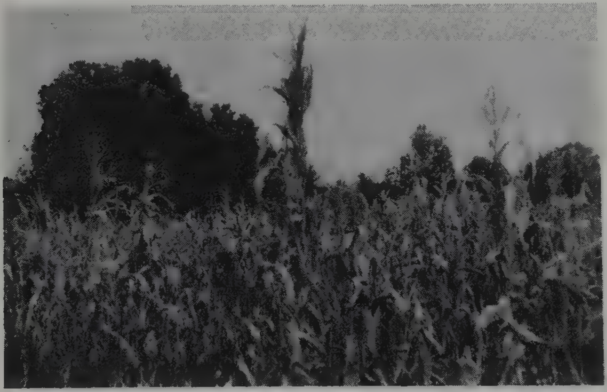

FIGURE 1. Corn field affected with crazy top, showing occasional tall plants exhibiting the giantism symptom.

In 1954 only a small number of plants in one field were found infected; however, in 1959, when the disease was next found, a number of widely separated fields along the eastern side of the State were infested. At State College several fields along a creek bottom were rather heavily infested and damage was severe. In an area of about 5 to 10 acres in one field, 90% of the plants were infected and most were completely barren. Only 15 to 20% of the plants were infected in other fields. In 1960 and 1961, additional affected fields, including a new area in the western part of the State, were observed.

The disease was primarily limited to the lower, wetter areas and often appeared to follow along creeks and drainage ditches. Most affected areas had been flooded by heavy spring rains when the plants were 4 to 8 inches tall, and others appeared to be subject to flooding but its occurrence was not certain. Apparently flooding might aid in germination of oospores and dissemination of the fungus rather than predispose plants to infection.

Though crazy top seems to be on the increase in Mississippi, because of the environmental limitations associated with dissemination and infection there appears to be little danger of its becoming economically important.

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[1] Cooperative investigations of Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Mississippi Agricultural Experiment Station. Journal Article 969 (New Series).

[2] Pathologist and Agronomist, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

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THE EFFECTS OF STALK ROT ON CORN YIELDS IN ILLINOIS

A. L. Hooker and M. P. Britton[1]

Abstract

Direct yield losses attributable to corn stalk rot were measured in farmers' fields over a 2-year period in Illinois. Differences in grain weight between diseased and adjacent healthy plants were used to calculate yield losses on an individual diseased-plant basis. These losses averaged 16.2% in 680 comparisons and ranged from 0.0 to 26.2% in the fields sampled. Using these data in relation to the incidence of disease, statewide losses were estimated to be 8.6% valued at $70,625,000 annually.

INTRODUCTION

On October 17, 1958 the authors were requested to examine a corn field in east-central Illinois. The field had not been harvested and 85% of the stalks were lodged. An examination of the plants revealed that most of the lodging was due to stalk rot. Infections by both Diplodia zeae (Schw.) Lév. and Gibberella zeae (Schw.) Petch. were evident. The paired-plant technique followed by Gaskin and Ullstrup (3) to estimate the effects of corn smut on yield was used in harvesting ears from diseased plants and adjacent healthy plants from 10 locations in the field. Ears from the diseased plants weighed 35% less than ears from the healthy plants. This experience suggested a more extensive examination of the effects of stalk rot on plant yield. Data obtained in 1958 from 17 fields in Champaign County, Illinois and in 1959 from 51 fields distributed throughout the State are presented in this paper.

LITERATURE REVIEW

Stalk rots are the most serious and widespread diseases of dent corn in Illinois and in the corn belt. In Illinois Diplodia zeae and Gibberella zeae are the most common pathogens involved (4, 5). These diseases are responsible for direct losses in yield due to the premature dying of infected plants and cause much of the lodging in corn. Lodged plants are difficult to harvest, many ears are missed and those in contact with moist soil are soon destroyed by fungi. In Minnesota in 1956 the losses caused by stalk rots were estimated to be 10% of the value of the crop (1). Koehler (4), following his extensive study of corn stalk rot, considered an average annual loss of 7 to 10% to be valid for Illinois. He cited data drawn from five yield tests, each test containing 60 or 81 hybrids. The acre yields following hand harvesting were averaged for the 10 hybrids with the lowest stalk rot rating and the 10 with the highest rating in each of the five tests. The average difference in yield between the two groups was 10 bushels or an approximate difference in yield of 10% in favor of the hybrids with the least stalk rot. Michaelson and Christensen (6, 7) inoculated two hybrids with D. zeae and with G. zeae over a 3-year period and measured yield reductions attributable to infection of 9.7% and 6.8%, respectively. Troyer (8), working with five hybrids in Illinois, measured yield reductions averaging 2.3% when D. zeae was inoculated in one internode and of 5.4% when inoculations were made in each of three internodes. These yield reductions were highly significant statistically. Koehler (4) also found that losses were greater if multiple inoculations were made.

MATERIALS AND METHODS

Samples for this study were obtained from commercial hybrids in farmers' fields at or near harvest over a 2-year period.

In 1958, 17 fields in Champaign County, Illinois were sampled. In each field the incidence of diseased-standing plants and of diseased-lodged plants was determined in four replications of 50 plants each. Plants were considered healthy if the stalks were green and firm. Diseased stalks were dead and brown in color. If they were bent or broken below the ear with an angle of 90 degrees or more they were classified as lodged. Ears were harvested from three plants: 1) healthy, 2) diseased-standing, and 3) diseased-lodged at 10 locations in each field examined. These plants were in close proximity to each other and were selected from areas of the field having uniform plant stands. The harvested ears from plants of the three classes were tagged and weighed to the nearest gram. They were then dried to a uniform moisture and shelled. Shelled grain weight and was obtained for the entire ear as well as for a hundred-kernel sample for each ear.

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In 1959, 51 fields throughout the State were sampled. Most of these were single, widely-separated fields distributed among eight of the nine crop reporting areas of the State. Sampling procedures were similar to those followed in 1958, but no attempt was made to separate diseased-standing from diseased-lodged plants. Ears were harvested from 10 pairs of healthy and diseased plants in each field, dried to a uniform moisture, and weighed. No other weights were taken.

These data were used to calculate the percentage loss in yield per diseased plant and per field (Tables 2, 3).

RESULTS AND DISCUSSION

Incidence of Stalk Rot: In 1958, the occurrence of diseased-standing plants in the fields surveyed ranged from 18.5 to 82.5%, with an average of 52.6% (Table 1). The diseased-lodged plants ranged from 0.5 to 13.5%, with an average of 5.4%. If the classes are combined, the incidence of diseased plants ranged from 25.5 to 85.0% with an average of 58.0%. In 1959 the range was 4.0 to 91.5% and the average 40.6% (Table 3). These averages are slightly below a recent 10-year average reported by Koehler and Boewe (5).

Table 1. Percent of diseased-standing and diseased-lodged corn plants in 17 fields in Champaign County, Illinois in 1958.

Field no.	% of plants with stalk rot		
	Standing	Lodged	Total
1	2	3	4
1	18.5	7.0	25.5
2	25.5	2.5	28.0
3	29.0	2.5	31.5
4	44.5	0.5	45.0
5	43.0	6.5	49.5
6	42.5	10.0	52.5
7	48.0	7.5	55.5
8	55.0	3.0	58.0
9	60.0	2.0	62.0
10	50.5	13.5	64.0
11	61.5	3.0	64.5
12	60.0	6.5	66.5
13	65.0	5.2	70.2
14	64.5	9.5	74.0
15	74.0	1.5	75.5
16	70.5	8.5	79.0
17	82.5	2.5	85.0

1958 Yield Data: The collective harvested ear weights of the 10 diseased-standing or of the 10 diseased-lodged plants was less than the ear weight of the 10 healthy plants in each field (Table 2). These differences were significant in 14 of the 17 fields sampled. All fields considered, the losses were greater for lodged than for standing diseased plants. The loss in yield per diseased plant, all samples in a given field considered as a unit, ranged from 9.0 to 42.2%. Multiplying the loss per plant as measured in each field by the incidence of disease in each field gave field losses ranging from 4.9 to 20.7%, averaging 12.8%.

The weight of the dried shelled grain from diseased plants was also consistently less than from the healthy plants; significant in 14 fields. The magnitude of the differences were less, however, in shelled grain than in harvested ear weights; this indicates a higher moisture content of ears from healthy plants than from diseased plants. The loss in grain weight per diseased plant ranged from 5.6 to 43.5% and averaged 18.5% for diseased-standing and 26.9% for diseased-lodged plants. Field losses averaged 11.2% and ranged from 3.6 to 17.8%.

The weight of 100 kernels from diseased-standing and diseased-lodged plants was less than that from healthy plants in all cases. However, these differences were significant in only 9 of 17 fields. These data indicate that stalk rot losses are partially due to a reduction in weight of individual kernels. The importance of this factor was undoubtedly underestimated in this study since all of the poorly developed kernels on ears from diseased plants were not recovered

Table 2. Yield and yield-differences in grams between healthy and diseased plants in 17 corn fields surveyed for stalk rot in Champaign County, Illinois in 1958.

Field Number	Healthy Plants	Diseased-Standing Plants				Diseased-Lodged Plants				Total	Significance
	Weight	Weight	Difference	% Loss /Plant	% Loss /Field	Weight	Difference	% Loss /Plant	% Loss /Field	% Loss /Field	of Comparisons
1	2	3	4 (2-3)	5 (4÷2)	6[a]	7	8 (2-7)	9 (8÷2)	10[a]	11 (6+10)	12
						Harvested Ear Weight					
1	3074	2499	-575	18.7	3.5	2459	-615	20.0	1.4	4.9	**[b]
2	3716	2814	-902	24.3	6.2	2583	-1133	30.5	0.8	7.0	**
3	3144	2337	-807	25.7	7.5	2288	-856	27.2	0.7	8.2	**
4	2915	2313	-602	20.7	9.2	2402	-513	17.6	0.1	9.3	NS[c]
5	3419	2581	-838	24.5	10.5	2434	-985	28.8	1.9	12.4	**
6	2872	2394	-478	16.6	7.1	2393	-479	16.7	1.7	8.8	*[d]
7	3368	2439	-929	27.6	13.2	2380	-988	29.3	2.2	15.4	**
8	3016	2258	-758	25.1	13.8	2056	-960	31.8	1.0	14.8	**
9	2981	2570	-411	13.8	8.3	2463	-518	17.4	0.3	8.6	NS
10	3588	2811	-777	21.7	11.0	2075	-1513	42.2	5.7	16.7	**
11	1953	1677	-276	14.1	8.7	1568	-385	19.7	0.6	9.3	NS
12	2791	2134	-657	23.5	14.1	2054	-737	26.4	1.7	15.8	**
13	3245	2397	-848	26.1	17.0	2473	-772	23.8	1.2	18.2	**
14	3746	2823	-923	24.6	15.9	2549	-1197	32.0	3.0	18.9	**
15	3379	3074	-305	9.0	6.7	1988	-1391	41.2	0.6	7.3	**
16	2914	2169	-745	25.6	18.0	1998	-916	31.4	2.7	20.7	**
17	2923	2423	-500	17.1	14.1	2110	-813	27.8	0.7	14.8	**
						Dry Shelled Grain Weight					
1	2168	1794	-374	17.2	3.2	1756	-412	19.0	1.3	4.5	**
2	2531	2054	-477	18.8	4.8	1790	-741	29.3	0.7	5.5	**
3	2357	1788	-569	24.1	7.0	1765	-592	25.1	0.6	7.6	**
4	2167	1745	-422	19.5	8.7	1766	-401	18.5	0.1	8.8	NS
5	2487	1952	-535	21.5	9.2	1779	-708	28.5	1.9	11.1	**
6	2061	1706	-355	17.2	7.3	1658	-403	19.6	2.0	9.3	**
7	2377	1851	-526	22.1	10.6	1736	-641	27.0	2.0	12.6	**
8	2217	1739	-478	21.6	11.9	1540	-677	30.5	0.9	12.8	**
9	1996	1885	-111	5.6	3.4	1759	-237	11.9	0.2	3.6	NS
10	2532	2128	-404	16.0	8.1	1522	-1010	39.9	5.4	13.5	**
11	1415	1239	-176	12.4	7.6	1164	-251	17.7	0.5	8.1	NS
12	2099	1574	-525	25.0	15.0	1533	-566	27.0	1.8	16.8	**
13	2387	1813	-574	24.0	15.6	1775	-612	25.6	1.3	16.9	**
14	2757	2165	-592	21.5	13.9	1982	-775	28.1	2.7	16.6	**
15	2520	2337	-183	7.3	5.4	1423	-1097	43.5	0.7	6.1	**
16	2125	1660	-465	21.9	15.4	1523	-602	28.3	2.4	17.8	**
17	2214	1864	-350	15.8	13.0	1591	-623	28.1	0.7	13.7	**
						Weight/100 Kernels					
1	301	267	-34	11.3	2.1	259	-42	14.0	1.0	3.1	NS
2	355	291	-64	18.0	4.6	281	-74	20.8	0.5	5.1	**
3	317	275	-42	13.2	3.8	262	-55	17.4	0.4	4.2	*
4	320	260	-60	18.8	8.4	281	-39	12.2	0.1	8.5	**
5	296	274	-22	7.4	3.2	260	-36	12.2	0.8	4.0	*
6	298	282	-16	5.4	2.3	258	-40	13.4	1.3	3.6	NS
7	340	285	-55	16.2	7.8	281	-59	17.4	1.3	9.1	**
8	301	252	-49	16.3	9.0	257	-44	14.6	0.4	9.4	**
9	294	261	-33	11.2	6.7	262	-32	10.9	0.2	6.9	NS
10	366	277	-89	24.3	12.3	260	-106	29.0	3.9	16.2	**
11	254	249	-5	2.0	1.2	231	-23	9.1	0.3	1.5	NS
12	301	285	-16	5.3	3.2	279	-22	7.3	0.5	3.7	NS
13	333	300	-33	9.9	6.4	269	-64	19.2	1.0	7.4	**
14	368	283	-85	23.1	14.9	280	-88	23.9	2.3	17.2	**
15	287	271	-16	5.6	4.1	242	-45	15.7	0.2	4.3	NS
16	274	254	-20	7.3	5.1	238	-36	13.1	1.1	6.2	NS
17	305	291	-14	4.6	3.8	271	-34	11.1	0.3	4.1	NS

[a] The percent loss per field was obtained by multiplying the percent loss per plant of each disease class by the number of plants in that class shown in Table 1.

[b] ** - significant at 1% level

[c] NS - not significant

[d] * - significant at 5% level

Table 3. Percentage of diseased plants, yield, and yield-difference in grams between healthy and diseased plants in 51 fields surveyed in Illinois in 1959.

Field No.	% Plants With Stalk Rot	Dry Ear Weight			% Loss /Plant	% Loss /Field
		Healthy	Diseased	Difference		
1	2	3	4	5 (3-4)	6 (3÷5)	7 (6x2)
1	4.0	2376	2111	-265	11.1	0.4
2	6.0	2566	2394	-172	6.7	0.4
3	6.5	2709	2314	-395	14.6	0.9
4	6.5	1810	1666	-144	8.0	0.5
5	8.5	2178	1927	-251	11.5	1.0
6	10.5	2767	2307	-460*[a]	16.6	1.7
7	14.5	1913	1835	-78	4.1	0.6
8	17.0	2506	2110	-396	15.8	2.7
9	17.5	2902	2479	-423	14.6	2.6
10	18.0	2772	2461	-311	11.2	2.0
11	18.0	2951	2197	-754**[b]	25.6	4.6
12	23.0	2646	2289	-357	13.5	3.1
13	24.0	2038	2066	+28	+1.4	+0.3
14	24.5	2798	2246	-552*	19.7	4.8
15	26.0	3114	2890	-224	7.2	1.9
16	27.0	2800	2146	-654**	23.4	6.3
17	28.0	2564	2084	-480	18.7	5.2
18	30.0	2936	2369	-567**	19.3	5.8
19	31.0	2921	2746	-175	6.0	1.9
20	33.0	2406	2123	-283*	11.8	3.9
21	35.5	2873	2647	-226	7.9	2.8
22	36.5	3078	2272	-806**	26.2	9.6
23	36.5	2721	2310	-411*	15.1	5.5
24	37.0	2666	2231	-435*	16.3	6.0
25	37.0	2601	2507	-94	3.6	1.3
26	38.0	3069	2534	-535**	17.4	6.6
27	38.0	2906	2642	-264*	9.1	3.5
28	38.5	2568	2516	-52	2.0	0.8
29	38.7	2898	2726	-172	5.9	2.3
30	39.5	3311	2835	-476**	14.4	5.7
31	41.5	2678	2302	-376	14.0	5.8
32	45.0	2297	2063	-234	10.2	4.6
33	46.0	2964	2566	-398	13.4	6.2
34	46.5	2485	1772	-713**	28.7	13.3
35	47.5	2766	2359	-407	14.7	7.0
36	53.5	2285	2296	+11	+0.05	+2.7
37	54.5	3142	2661	-461*	14.7	8.0
38	56.5	2913	2332	-581**	19.9	11.2
39	57.5	2447	2384	-63	2.6	1.5
40	58.0	2545	2240	-305	12.0	7.0
41	59.5	2217	2035	-182	8.2	4.9
42	60.0	2868	2461	-407*	14.2	8.5
43	65.0	2835	2638	-197	6.9	4.5
44	67.5	2787	2281	-506*	18.2	12.3
45	70.3	2889	2333	-556	19.2	13.5
46	73.5	2382	2146	-236	9.9	7.3
47	74.5	3243	2442	-801**	24.7	18.4
48	76.5	2848	2630	-218	7.7	5.9
49	87.5	2399	2250	-149	6.2	5.4
50	90.0	2108	1721	-387	18.4	16.6
51	91.5	2266	1853	-413*	18.2	16.7

[a] Significant at 5% level

[b] Significant at 1% level

during the mechanical shelling operation. Even so, 66% of the field losses could be attributed to a reduction in weight of individual kernels.

When all fields and classes of plants are considered in a combined analysis of variance, differences between diseased-standing plants and healthy plants and between diseased-lodged plants and healthy plants were highly significant for harvested ear weights, for dry shelled grain weight, and for hundred-kernel weight. In these comparisons, variances associated with lodged plants were about twice as great as those associated with standing-diseased plants. Differences among fields were statistically significant for harvested ear weight and for dry shelled grain weight but not for hundred-kernel weight, suggesting that differences in total yield among the 17 fields were due more to the number of kernels produced than to the size of kernels. The loss per diseased plant was not consistent from field to field; a significant treatment x field interaction was measured. This is also evident from an inspection of the figures in columns 5 and 9 of Table 2. The magnitude of the loss per diseased plant was not associated with disease incidence. Field losses, however, tended to be greater as the incidence of disease increased. The variance due to treatments (three classes of plants) was approximately 10 times greater than the summation of the variances due to fields, treatments x fields, and random variation. The coefficients of variability were 18.83, 18.27, and 13.91% for harvested ear weight, dry shelled grain weight, and hundred-kernel weight, respectively. Of the three units of comparison used in 1958, dry shelled grain weight was believed to be the most critical as it eliminated moisture differences and took into account both kernel size and number.

1959 Yield Data: In all but two fields, the ears from diseased plants weighed less than those from healthy plants (Table 3). In 19 of the fields these differences were statistically significant. This proportion was below that experienced in 1958 and may have been due in part to the severe drought prevailing in east-central Illinois in 1959. Samples were also taken from several fields located in a region where a severe epiphytotic of northern leaf blight (*Helminthosporium turcicum* Pass.) prevailed. The incidence of stalk rot was very high in these fields and often only plants with poor ear development remained free from stalk rot. Even though these conditions prevailed in a number of fields, the average loss per diseased plant was 13.2% and the average loss per field was 6.1% when all 51 fields were considered. In the 19 fields where statistically significant differences in ear weight between healthy and diseased plants were measured, the average loss per diseased plant was 18.6% and the average field loss was 8.0%.

When data from all fields were combined most of the variation was due to the differences in yield between the healthy and diseased plants. Differences among fields and the interaction of fields x treatments, although small, was also statistically significant.

In fields 10, 17, 23, 25, 32, 34, and 45 and in fields 19, 27, 29, and 37 different hybrids were growing in close proximity to each other under essentially the same environmental conditions. In the the first group the loss per diseased plant ranged from 3.6 to 28.7% and field losses ranged from 1.3 to 13.3%. In the second group the loss per plant ranged from 5.9 to 14.7% and field losses from 1.9 to 8.0%. The incidence of stalk rot varied among these hybrids reflecting different degrees of genetic resistance to the disease. In addition, losses per diseased plant also differed greatly among these hybrids.

Inferences: Critical quantitative data on crop losses due to stalk rot are difficult to obtain mainly because of difficulty in isolating the effects of the disease from other factors affecting yield.

A determination of the incidence of diseased plants in a field does not give a reliable estimate of the losses caused by stalk rot. The multiplication of incidence of disease by average yield reduction of individual diseased plants is useful but is far from satisfactory.

If both years are considered and data for dry shelled grain in 1958 is used, the average reduction in yield per diseased plant for stalk rot was 16.2%. Field losses for both years averaged 8.6%.

The losses are direct reductions in yield due to premature killing of the plant and assume that all ears produced are harvested. Additional losses occur in the form of unharvested ears, ear rots, and harvest difficulties. Thus, an 8.6% loss due to stalk rot is a conservative estimate and results in a yield reduction of 46,463,804 bushels per year in Illinois, based on the most recent 10-year average of corn production in the State. Based on the average price per bushel for the same 10-year period, this loss would be valued at $70,624,982 which is approximately $531 for every farmer growing corn in Illinois. To look at it another way, in the absence of stalk rot, the average Illinois corn crop could be produced on 775,172 fewer acres. Since it costs approximately $65 per acre to produce corn in Illinois, this results in the expenditure of $50,386,180 from which no returns are obtained. Net return per acre of corn grown in Illinois could be increased by approximately 28% if losses due to stalk rot were eliminated.

Losses due to stalk rot in corn in Illinois are several times greater than losses due to some plant diseases which may develop in epiphytotic proportions in certain years and thereby receive widespread attention. For instance, the most severe crown rust epiphytotic on oats in 36 years occurred in Illinois in 1957. Losses were reliably estimated to be $16, 900, 000 (2) which is less than one-fourth the calculated annual loss from corn stalk rot. Stalk rot losses are not fully appreciated because they occur more or less continually year after year, with little fluctuation in severity.

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ADDITIONAL SOURCES OF RESISTANCE TO PUCCINIA SORGHI IN THE UNITED STATES[1]

A. L. Hooker[2]

Abstract

Twenty-six additional sources of resistance to Puccinia sorghi have been obtained from Australia, Africa, Guatemala, Turkey, Uruguay, Yugoslavia, and the United States. These corn strains expressed chlorotic to necrotic flecks or mixtures of necrotic spots and small uredia when seedlings were inoculated with one or more American cultures of P. sorghi. Several strains were highly resistant to numerous cultures of the fungus.

INTRODUCTION AND LITERATURE REVIEW

Hooker and Le Roux (1) reported 64 corn strains that expressed resistance to Puccinia sorghi Schw. in the seedling stage. Kramer and Ullstrup (4) added the Plant Introductions P. I. 163597 from Guatemala and P. I. 167388 and P. I. 185054 from Turkey to the list of resistant corn strains.

Since 1957 corn workers from several countries have exchanged seed of rust resistant corn strains with the author (3). In addition, other resistant types have been obtained by empirical screening methods. This report summarizes these rust tests and enumerates 26 additional sources of resistance to American cultures of P. sorghi.

MATERIALS AND METHODS

Seed stocks of inbreds were increased by selfing several plants and open pollinated varieties were increased by sib-pollinating 40 or more plants of each variety.

Seedling populations of 163 inbred lines and 44 open-pollinated varieties were evaluated for reaction to one or more cultures of P. sorghi in the greenhouse. In initial tests, five seedlings of each inbred line were tested; in open pollinated varieties 120 seedlings from sib-pollinated plants or 20 seedlings from each of 10 or more self-pollinated plants were tested. Seedlings were inoculated in the greenhouse by dusting with urediospores of P. sorghi diluted with talc and holding in a saturated atmosphere for 15 hours.

For later studies, inbred lines uniform for rust reaction were established through selfing and progeny testing. Where the frequency of resistant seedlings in a variety was low, large populations of seedlings were retested in late spring and the resistant plants transplanted in the field for selfing. Those rust-resistant strains that were difficult to maintain in the corn belt were crossed with corn belt rust-susceptible inbred lines and then selfed to produce resistant inbred lines.

RESULTS AND DISCUSSION

Seedlings of several corn strains (Table 1) were resistant to P. sorghi. Rust resistant seedlings expressed chlorotic to necrotic flecks or mixtures of necrotic lesions and small uredia. Several inbred lines were uniform for rust reaction; the open-pollinated varieties usually segregated for resistance to P. sorghi.

Several of the strains were not only highly resistant but also were resistant to a majority of the rust cultures used. For example, among 29 of the rust cultures used in these tests corn strains P. I. 213777, Njoro Flat White, B216, B217, P. I. 163558N, P. I. 186191, 25, M16, NN14, All Nations, 90, and E697 were resistant to 29, 28, 27, 27, 25, 24, 20, 20, 20, 18, 17 and 13 cultures, respectively. In contrast, inbred GJ39-25 was resistant to only 1 culture.

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Table 1. Corn strains resistant to Puccinia sorghi in greenhouse seedling tests.

Corn strain	Source
21A	Australia[a]
25	Australia[b]
90	U. S. - Illinois
All Nations	Australia[b]
B216	U. S. - Iowa[c]
B217	U. S. - Iowa[c]
E697	South Africa[d]
Gobi Yellow	Africa[a]
Kitale Flat White	Kenya[e]
M16	Australia[b]
Njoro Flat White	Kenya[e]
NN14	Australia[b]
P. I. 163558N	Guatemala[f]
P. I. 172597	Turkey[g]
P. I. 186191	Uruguay[g]
P. I. 213777	U. S. - S. Dakota[g]
P. I. 214297	U. S. - Illinois[g]
P. I. 217405	U. S. - Texas[g]
P. I. 217415	U. S. - W. Virginia[g]
P. I. 218186	U. S. - Arizona[g]
P. I. 218187	U. S. - Arizona[g]
P. I. 221845	South Africa[g]
P. I. 251653	Yugoslavia[g]
P. I. 251655	Yugoslavia[g]
Synthetic B	U. S.[c]
Teko Yellow	Africa[a]
TxGJ39-25	U. S. - Texas[b]

[a] Seed provided by L. M. Josephson, Tennessee Agricultural Experiment Station, Knoxville.

[b] Seed provided by K. S. McWhirter, New South Wales Department of Agriculture, Grafton.

[c] Seed provided by W. A. Russell, Iowa Agricultural Experiment Station, Ames.

[d] Seed provided by P. M. LeRoux, Faculty of Agriculture, University of Pretoria, Pretoria, Union of South Africa.

[e] Seed provided by M. N. Harrison (through H. C. Thorpe), Department of Agriculture, Kitale, Kenya.

[f] Seed provided by H. H. Kramer, Indiana Agricultural Experiment Station, Lafayette.

[g] Seed provided by W. H. Skrdla, Regional Plant Introduction Station, Ames, Iowa.

Segregates differing in rust reaction were often obtained from the same variety. They differed in infection type or showed differential reactions to a series of rust cultures. Three genetically different inbreds were obtained from the Introduction P. I. 163558.

The genes for rust resistance in these strains are being identified (2, 3, 5, 6) as well as being transferred to the rust susceptible inbreds B14 and R168 by backcrossing. How many of these are new genes to the Illinois collection remains to be determined.

It should be recognized that in studies of this type, genes for resistance can be detected only in terms of the genes for avirulence present in the rust cultures used. Many of the corn strains tested were susceptible. Conceivably, some of these strains may have genetic factors for resistance, as all genes for avirulence in P. sorghi may not have been represented in the rust cultures used.

The sampling methods employed, especially in the case of open-pollinated varieties, would disclose mostly dominant genes for resistance. Resistance due to duplicate or triplicate recessive genes (5) would rarely be measured. Such types of resistance are known (1, 5) and have been detected in tests of established inbred lines.

The frequency of genes for rust resistance in the seedling stage is low. Kramer and Ullstrup (4) evaluated 12-kernel samples from 1066 corn introductions and found only 16 strains to be segregating for resistance to P. sorghi. In the present investigation, 120-kernel samples from 36 corn varieties or synthetics were tested and only 70 resistant seedlings were identified. Forty of these were in one variety, but some varieties had as few as two resistant plants. In the seedling stage most of the established inbred lines were susceptible to rust.

Resistance to cultures of P. sorghi prevailing in the United States has been found in corn strains from widely separated geographical regions of the world. Presumably mutations for rust resistance in corn have survival value in the presence of the pathogen. Therefore, from an evolutionary point of view, either similar strains of the pathogen have prevailed in the Western Hemisphere, Australia, South America, Europe, and Africa or mutations for resistance occurred before corn was introduced from Central and North America to the other regions of the world.

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THE OCCURRENCE OF CYTOSPORA CANKER ON SPRUCE IN THE PRESENCE OF XIPHINEMA AMERICANUM AND CRICONEMOIDES XENOPLAX[1]

A. H. Epstein[2] and G. D. Griffin[3]

Surveys of Wisconsin's commercial tree nurseries for nematodes showed that the commonly grown forms of spruce (*Picea pungens*, *P. glauca densata*) in some plantings are extensively infected by a canker-producing organism which causes die-back of lower branches. This results in trees that are practically worthless for landscaping purposes and has caused severe loss in several nurseries.

Specimens of cankered material taken to the laboratory were found to contain the fruiting bodies of a *Cytospora* sp. which corresponds to that described by Waterman (2) as *Cytospora kunzei* Sacc. var. *piceae* Waterman. The Valsa stage, however, was not found (Fig. 1).

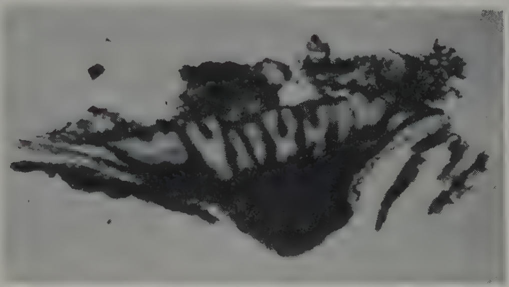

FIGURE 1. Cross section of a pycnidial stroma.

According to observations by Hawksworth and Hinds (1), Waterman (2) and Wright (3), Cytospora canker is more or less a secondary disease attacking trees weakened by some other agent such as drouth, fire and insects. Vigorously growing trees are thought not to be susceptible. The trees found infected in this study were all under 15 years of age and most were under 10. These trees should ordinarily be in a very vigorous state and not subject to attack by this fungus.

Soil samples collected from all sites in which the diseased trees occurred contained heavy populations of the dagger nematode, *Xiphinema americanum* Cobb, and a ring nematode, *Criconemoides xenoplax* Raski. The trees were in obviously poor condition with considerable yellowing of the new shoots and annual growth was much reduced. The root systems were severely damaged and devoid of feeder rootlets. Mortality of trees transplanted from such areas ranged as high as 78%. Tests to determine the pathogenicity of the nematodes from these areas are under way.

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STALK ROT OF CORN IN THE EGYPTIAN REGION

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Stalk rot of corn was of no importance in the Egyptian Region and no epidemics occurred until the early 1950's. However, the disease was found later in many localities, forming epidemics and causing great losses. This can be attributed to the distribution of hybrid corn, as epidemics were associated with the production of hybrid corn. In this period, a vast program for the production of hybrid corn was initiated and hybrids were distributed to the farmers. Losses up to 30% of the total yield are estimated (2, 3).

Symptoms of infected plants vary from yellowing or death of a few leaves to complete death of the plant. When stalks are cut longitudinally near the base, rotting of the stalk appears as discoloration or complete disintegration of the tissue except for the vascular bundles. In some cases sclerotia may be present and the tissue turns black in color. In some other cases the tissues are soft and juicy, owing to the presence of bacterial fluids.

Stalk rot in the Egyptian Region is caused by several pathogens. Sabet in 1954 (5) reported a bacterial stalk rot caused by Erwinia carotovora f. sp. zeae Sabet. Work at the Bahtim Experimental Station since 1956 (2, 3) has shown that stalk rot is also caused by Fusarium spp. or by Sclerotium bataticola. These fungi were isolated from plants rotted in different degrees, purified, then artificially infected in corn plants either planted in the field or in the greenhouse by the method used by Young (6).

FACTORS AFFECTING SEVERITY OF THE DISEASE

Since 1956, studies conducted at the Bahtim Experimental Station have shown that many factors can affect the severity of the disease in the Egyptian Region:

(a) Location: From a study done in 1958, it was found that stalk rot incidence increases more in the northern part of the Delta (Alexandria) than in the southern part (Bahtim). Corn plants are less infected in the central part of the Egyptian Region (Matai, Minia Province) (2, 3).

(b) Fertilizers: 1 -- Addition of high levels of nitrogen mostly tends to increase incidence of the disease. 2 -- Different levels of potassium or phosphorus have no or little effect on percentage of infected plants (4).

(c) Defoliation and detasseling, an agricultural practice employed by some Egyptian farmers as source of green fodder for animals in the summer, increase incidence of the disease (2).

(d) Date of sowing: More plants are infected if corn is planted in the period from June 5 to August 15, but less plants are infected if corn is planted prior to or after this period (1).

Breeding for stalk rot resistance has been included in the production of hybrid corn. Some inbred lines at the Bahtim Experimental Station are highly resistant to this disease complex and have been incorporated recently into the breeding program.

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ATTEMPTS TO ELIMINATE SWEETPOTATO INTERNAL CORK VIRUS BY HEAT TREATMENTS

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Sweetpotato sprouts and mother roots of variety Porto Rico affected with internal cork virus (ICV) were subjected to various heat treatments in attempts to eliminate ICV. This work was started about 1949 before methods were available for freeing sweetpotatoes of ICV. Although none of the treatments was effective, a brief summary of the results is presented for records on heat tolerance of sweetpotato.

ICV was assayed in all experiments by examining for internal cork lesions in sweetpotato roots produced by treated sprouts or by sprouts from treated mother roots. Preliminary results showed that sweetpotato sprouts and roots would not survive even 5-minute exposures in hot water at 54° C. Further experiments showed that sprouts or vine cuttings, placed in 10-inch diameter glass chambers lined with moist paper, survived exposure for 1 hour in an oven at 55°. Assays for ICV showed that the virus apparently was not affected by the following exposures of such sprouts and cuttings: 1) 43.5° C for 24 to 48 hours, 2) 50° for 1 to 2 hours, 3) 55° for 1 hour.

Table 1. Hot-air treatment of ICV-infected Porto Rico variety sweetpotato roots in a 55° C oven. 1957.

Treatment	Diameter of roots (inches)	Time in oven (hours)	Root temperatures (° C)	Mean number roots surviving 5 months after treatment[a]
A	2-3	0	24°	15
B	2-3	6	Over 38° for 6 hours 48°-51° for 4 hours	9
C	2-3	8	Over 38° for 8 hours 49°-55° for 5 hours Over 51° for 2 hours	5
D	2-3	9 1/2	Over 38° for 9 hours Over 49° for 8 hours 53°-55° for 5 1/2 hours	1
E	1-2	0	24° C	18
F	1-2	6	Over 46° for 4 1/2 hours 51°-52° for 2 hours	2
G	1-2	8	Over 49° for 8 hours 52°-55° for 6 1/2 hours	0
H	1-2	9 1/2	Over 49° for 9 hours 53°-55° for 8 hours	0
K	2-3	0	24°	19
L	2-3	6	Over 45° for 4 1/2 hours 50°-51° for 2 hours	9
M	2-3	8	Over 49° for 6 1/2 hours 53°-54° for 4 hours	3
N	2-3	9 1/2	Over 49° for 7 1/2 hours 52°-55° for 5 hours 55° for 1 hour	3

[a] Mean of 6 replicates of 30 roots for treatments A-D and of 4 replicates of 30 roots each for treatments E-N.

Additional experiments were made in an electric oven, used at Louisiana State University for treating sugarcane stalks to control ratoon stunting disease, and described in detail in the Sugar Bulletin 33: 243 (1955). Sweetpotato roots infected with ICV were used. Temperatures near the center of roots were determined by use of thermocouples and a potentiometer. A small percentage of the roots survived exposures in the oven at 54° to 55° C for 9 1/2 hours (Table 1). Temperatures inside the roots were below 54°, however, for all but a relatively short period of the exposure.

Sprouts from two roots of treatment K, from one of the surviving roots of treatment M, and from the three surviving roots of treatment N were planted under 32 x 32-mesh screen, known to exclude vectors of ICV. Roots from the plantings were examined for internal cork lesions 5 months after harvest (Table 2). Internal cork lesions were found in roots produced in plantings made with sprouts from treated roots (M and N) as well as from nontreated roots (K).

Table 2. Internal cork lesions on hot air-treated Porto Rico variety sweetpotato mother roots 5 months after harvest.

Treatments[a]	Number of roots examined	Cork-affected	% cork-affected
IK	5	3	60
IIK	11	8	73
IM	7	1	14
IN	3	2	67
IIN	2	0	0
IIIN	5	2	40

[a] For details on treatments, see Table 1.

It is indicated that sweetpotato roots and sprouts are less severely injured by hot air than by hot water. Since temperatures inside roots of treatment N were 55° C for at least an hour, the thermal inactivation point of ICV appears to be above that temperature, which is near the lethal point for sweetpotato tissue.

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ELIMINATION OF ROOT-KNOT NEMATODES FROM INFESTED SWEETPOTATO ROOTS AND PLANTS

W. J. Martin

Summary

Hot air treatment (50° C for 4 to 8 hours) of sweetpotato variety Goldrush mother roots was effective in eliminating root-knot nematodes (Meloidogyne incognita acrita) from infested roots. O,O-diethyl O-2-pyrazinyl phosphorothioate (Cynem) as a dip for sweetpotato plants at concentrations (active ingredient) of 1:1600, 1:2400, and 1:3200 for 12 hours eliminated root-knot nematodes from infested plants. The same concentrations at 1/2 and 2 hours appear to have greatly reduced nematode populations in treated plants.

INTRODUCTION

Losses are known to be considerable from root-knot nematode infestations in sweetpotatoes. Recently Krusberg and Nielsen (3) summarized the effects of root-knot nematodes on sweetpotato and reported on pathogenesis of these nematodes to the variety Porto Rico.

Preplanting soil fumigation was reported as effective in reducing losses from root-knot nematodes in sweetpotato (5, 6). However, even under best of soil fumigation practices, root-knot nematodes may increase toward the end of the growing season and infest the storage roots. Such infestations, late in the growing season, may not adversely affect yield or quality of that crop, but they may be extremely important in carrying-over the nematodes to succeeding crops if the infested roots are used as seed roots. Relatively few nematodes in infested seed roots may increase rapidly in sweetpotato plant beds, where temperatures ideal for plant production are also near ideal for nematode increase. Thus, a method of ridding sweetpotato roots and/or plants of root-knot nematodes is extremely valuable in any scheme for controlling these pests on sweetpotatoes.

Burk and Tennyson (1) reported a hot water treatment of 65 minutes at 116° F (46.7° C) for control of nematodes in sweetpotato seed roots. Varieties Nancy Hall and Porto Rico were used. In their experiments there was about 1/3 mortality of roots receiving hot water treatment at 119° F (48.3° C) for 50 minutes, and they stated that temperatures of 122° F (50° C) or higher were too injurious to sweetpotato root tissue to be used with any degree of success.

This paper reports results of hot air treatment of infested mother roots and chemical treatment of infested plants of variety Goldrush for elimination of root-knot nematodes, Meloidogyne incognita acrita Chitwood, 1949.

METHODS AND RESULTS

Hot Air Treatment of Mother Roots: Hot air was reported as being much less injurious than hot water to sugarcane stalks in treating for the ratoon stunting disease (8). Hot air appears less injurious than hot water to sweetpotato roots also (4).

Experiments to control root-knot nematodes in sweetpotato were made with roots 2 to 3 inches in diameter of variety Goldrush. All roots were heavily infested with nematodes. Forty roots per treatment were used. Roots for each treatment were placed in 50-pound crates and then into an oven stabilized at 49° to 50° C. Temperatures near the center of roots for the duration of treatments were recorded by use of thermocouples and a potentiometer (Table 1).

Treatments were made on January 26, 1959 and roots were kept at about 24° C after treatment. Two days after treatment four roots from each treatment were bedded in steam sterilized river silt, one root per 8-inch clay pot, and kept in a greenhouse at above 24°. All roots bedded in pots survived and produced sprouts which were allowed to grow until July. Seven months after treatment, plants were removed from the pots with as many attached roots as possible, and roots were rated for root-knot symptoms into 1 of 5 classes as follows: 1=none, 2=trace, 3=slight, 4=moderate, and 5=severe. At the same time, 1 pint of soil was taken from each pot with a soil sampling tube. The nematodes were screened from this soil and the root-knot nematode larvae were counted (Table 1). The 36 nonbedded roots from each treatment were kept in storage at 22° to 24° for 7 months and then the number of sound roots was determined (Table 1).

Table 1. Hot air treatment of root-knot nematode infested Goldrush variety sweetpotato roots in a 49° -50° C oven. January 1959.

Treatment[a]	Time	Temperature inside root (° C)	Root-knot ratings 7/28	No. larvae/ pint soil 7/28	No. of 36 roots surviving 6 months' storage
A-Nontreated		25.5	4.0	13,670	29
	8:15 a.m.	25.5			
	8:30	26.7			
	9:30	35.6			
	10:15	37.8			
	10:30	38.9			
B-Removed	11:15	43.3	3.0	10,060	33
C-Removed	12:15 p.m.	45.6	3.5	1,810	30
D-Removed	1:15	46.7	1.3	370	28
E-Removed	2:15	48.9	1.0	300	31
	3:15	48.9			
F-Removed	4:15	49.4	1.0	0	25
G-Removed	6:15	49.4	1.0	50	17

[a] All roots were placed in the oven at 8:15 a.m. Roots of the different treatments were removed at the time indicated for each.

The small numbers of nematode larvae found in soil from treatment G may have increased from a few nematodes surviving the heat treatments. However, it is more likely that they were the result of increase from an accidental introduction of a small number of larvae during watering of the pots in the greenhouse over the 5-month growing period. Inside root temperatures in treatment G were above 49° C for over 4 hours, and it is doubtful that root-knot nematodes survived this exposure. In treatment C, where temperatures near the center of the root were 43° to 46° for about 1 hour, a high percentage of the nematodes were killed. No doubt temperatures near the surface of the root, where most nematodes eggs are found in sweetpotatoes, were closer to 50° than to 43° to 46°.

The results indicate that placing sweetpotato roots of 2 to 3 inch diameter in a hot air oven at 50° C for 4 to 8 hours will eliminate most, if not all, of the root-knot nematodes in roots without significantly affecting the keeping qualities of the treated roots.

Chemical Treatment of Plants: Plants of variety Goldrush heavily infested with root-knot nematodes (*M. incognita acrita*) were used in these experiments. In 1960 an experiment was made using 1,6-hexanedithio diacetate (Shell SD 4965). Roots of the infested plants were kept in solutions containing 100, 200, and 400 ppm of SD 4965 for 8, 12, and 24 hours. Treatments were replicated four times with each treatment replicate consisting of five plants each, planted in sterilized soil in 6-inch clay pots. After 3 months roots of the plants were rated for root-knot symptoms. Treated and nontreated plants were all moderately to heavily infested with root-knot nematodes.

In 1961, a similar experiment was made using *O,O*-diethyl *O*-2-pyrazinyl phosphorothioate (Cynem) at concentrations of 1:1600, 1:2400 and 1:3200 for 1/2, 2, and 12 hours. Roots and about 3 to 4 inches of the plant stems were emersed in the solutions. Treatments were replicated four times with each treatment replicate consisting of four plants in a 6-inch clay pot containing sterilized river silt and planted immediately after removal from the solutions. The dip solutions were made by using the following amounts of a 45% solution of Cynem in 800 cc of water: 1.1 cc for the 1:1600 solution, 0.7 cc for the 1:2400 solution, and 0.55 cc for the 1:3200 solution. The experiment was started on July 7, 1961 and terminated on October 12, 1961. Within a week after planting there was evidence of slight injury (dying back of leaf tips) in plants treated for 12 hours in the 1:1600 and 1:2400 solutions. Two weeks later three plants treated for 12 hours in 1:1600 solution and two plants treated for 12 hours in 1:2400 solution had died. After 24 days the remaining plants receiving the above two treatments had recovered and were growing normally, and by the end of the experiment they had made better growth than the nontreated plants.

The results show that treatment for 12 hours in the three concentrations of Cynem was effective in eliminating root-knot nematodes (Table 2). The data show that the 1/2 and 2-hour exposures to these solutions appear to have drastically reduced nematode populations. Further work is warranted on proper concentration-timing for determining the most effective treatment

Table 2. Treatment of root-knot nematode infested Goldrush variety of sweetpotato plants with solutions of Cynem. Results taken 3 months after treating.

Treatment Concentration	Time (hours)	Root-knot rating[a]	No. surviving plants	No. larvae per pint of soil
1:1600	1/2	3.95	16	4,030
1:1600	2	2.10	15	200
1:1600	12	1.00	13	0
1:2400	1/2	1.58	16	4,550
1:2400	2	1.78	16	1,420
1:2400	12	1.08	14	0
1:3200	1/2	2.23	16	2,480
1:3200	2	2.15	16	1,440
1:3200	12	1.20	16	0
None-check		4.35	16	16,240

[a] Mean of four replicates of four plants each. Plants were rated into 1 of 5 classes as follows: 1=none, 2=trace, 3=slight, 4=moderate, 5=severe.

for eliminating root-knot nematodes from infested sweetpotato plants with Cynem. There are several recent reports of the effectiveness of Cynem in controlling nematodes in plant roots (7, 9, 10). This, however, is the first report of the effectiveness of Cynem in controlling nematodes in sweetpotato plants.

The roots and plants used in these experiments were infested with M. incognita acrita. It is known that other species of root-knot nematodes may infest sweetpotatoes (2). The reaction of these other species to such treatments has not been determined.

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PATHOGENICITY OF THE STUBBY ROOT NEMATODE TO ONION

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Although the occurrence of Trichodorus christiei Allen, the stubby root nematode, is reported to be widespread, especially in the southeastern part of the United States (1, 2), and the species has been shown experimentally to have a wide host range (3), none of the data available in the literature have definitely shown a pathogenic association between this nematode and onion.

This paper presents data demonstrating that T. christiei is pathogenic to onion and contains descriptions and photographs of the symptoms produced in this host.

Field observations during the summer of 1960 in the muck lands of New York revealed a hitherto undetermined syndrome in onions. A general stunting of the plant was coupled with a die-back of the terminal portions of the leaves and a general yellowing progressing downward from the leaf tips. The root system was small; not only were the roots unusually short but they were also abnormally branched and, in most cases, slightly swollen just above the root tip.

Because of the similarity between these symptoms and the previously described symptoms caused by T. christiei in other hosts, soil was obtained from fields where the disease occurred and processed by the Seinhorst elutriator; the nematodes in the elutrate were counted and identified. T. christiei was the only plant pathogenic nematode present.

The remaining soil was divided into two equal portions; one portion was then fumigated with the equivalent of 40 gallons of 1,3-dichloropropene (Telone) per acre, whereas the other served as a control. After 2 weeks, the soil was potted in 4-inch clay pots, each of which was plunged in fine silica sand in a gallon crock (Fig. 1). Onion seeds of the variety Ebenezer were planted in each pot. The treatment and the control were each replicated 32 times.

In another experiment, four onion plants in each of two pots of steam-pasteurized soil (determined to be free from plant parasitic nematodes) were inoculated with T. christiei, 50 individuals being added to each pot. The nematodes were transferred with a needle into water in a Syracuse watch glass and then pipetted into the soil near the roots.

At the end of 70 days, the plants in both experiments were harvested and their fresh weights determined (Table 1); symptoms identical with those observed in the field developed in plants growing in the nonfumigated soil of the first experiment and also in those growing in the steam-pasteurized soil infested with T. christiei in the second experiment (Figs. 2, 3). Plants grown in fumigated soil were normal. Steam pasteurized soil, like that used in the second experiment, is used routinely for growing onions; symptoms like those described here have never been observed. T. christiei, from 53 to 287 individuals per pot, were present in the nonfumigated soil whereas no plant pathogenic forms were present in the fumigated soil.

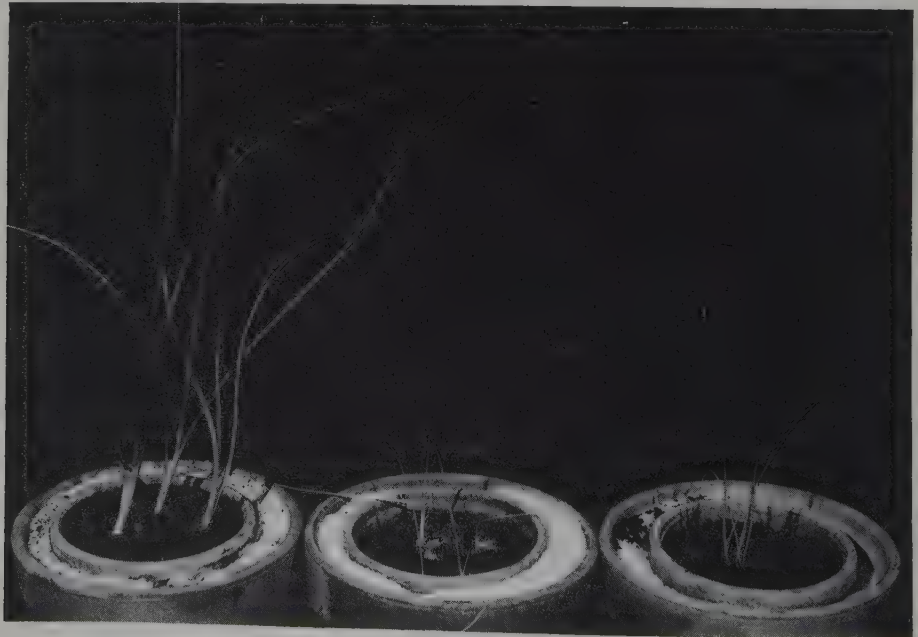

FIGURE 1. Onion plants growing in soil naturally infested with T. christiei (center), the same soil fumigated with Telone (left), and in another soil steam-pasteurized and then infested with T. christiei.

Table 1. Root and top weights of onions grown in soil naturally infested by Trichodorus christiei, in the same soil fumigated with Telone, and in another muck soil steam pasteurized and then infested with T. christiei.

Soil	Treatment	Average fresh weight (g/plant) tops	roots
Naturally infested	Nonfumigated[a]	0.13	1.18
	Fumigated[a]	2.47	5.99
Steam pasteurized	Infested with T. christiei (50/pot)[b]	0.11	1.02

[a] Replicated 32 times.

[b] Replicated twice, four plants per replicate.

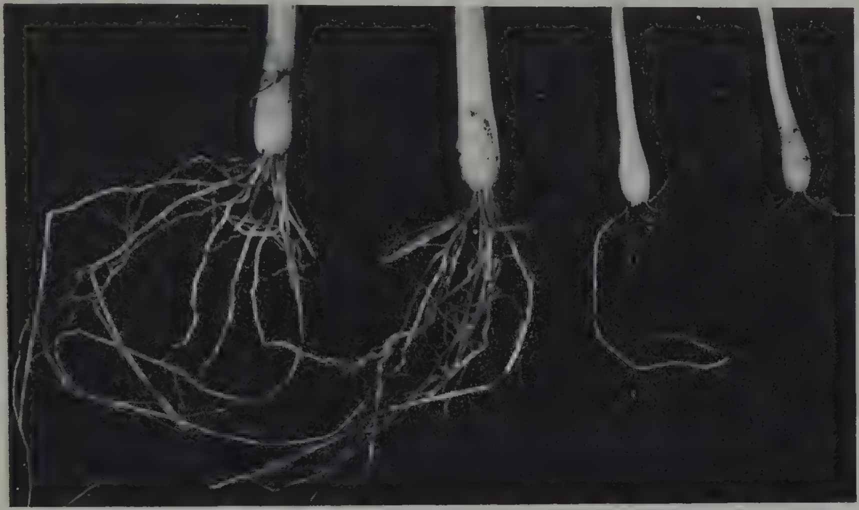

FIGURE 2. Onion roots from soil naturally infested with T. christiei (right), and in the same soil fumigated with Telone (left).

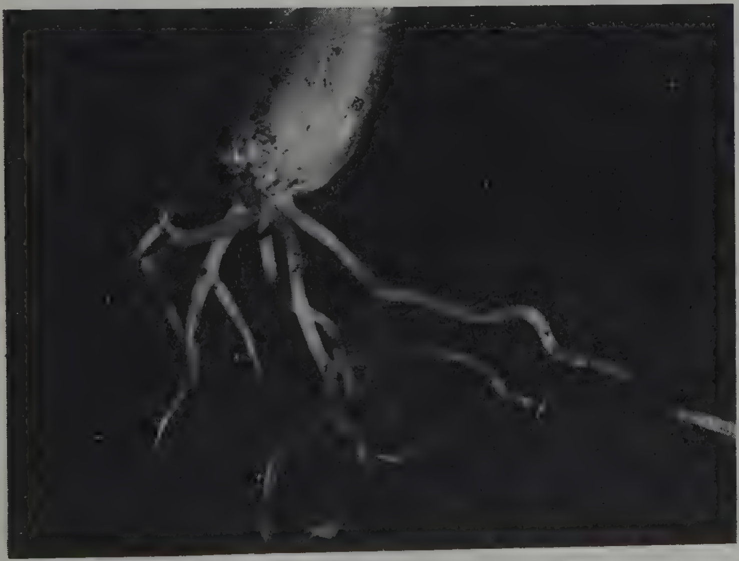

FIGURE 3. Close-up of onion roots grown in soil infested with T. christiei.

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INTERFERTILITY STUDY OF POLYPORUS PALUSTRIS AND OTHER BROWN ROT SPECIES OCCURRING ON CONIFERS AND HARDWOODS

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Polyporus palustris Berk. & Curt. has been collected frequently in several southern States on pine stumps and dead wood of coniferous trees containing brown rot. Overholts (11) reports it from South Carolina, Georgia, Florida, Louisiana, Mississippi, and probably Arkansas. He considers it to be entirely southern in distribution and to be limited to coniferous trees. He doubts that it could occur in New York State as reported by Lowe (3).

Polyporus durescens Overh. was described as occurring on logs and stumps of deciduous trees with the type specimens from Ohio and citations from New York, Pennsylvania, Louisiana, Kentucky, and Indiana (10). Overholts distinguishes P. durescens from specimens considered to be Polyporus spraguei Berk. & Curt. only by microscopic study. The basidia of P. spraguei are larger and the spores globose rather than cylindrical as in P. durescens. P. spraguei and P. durescens are both associated with a similar type of brown rot occurring on hardwood species. When discussing P. durescens, Overholts (11) states that "specimens of P. palustris sometimes appear quite similar but this species occurs only on wood of coniferous trees and is entirely southern in distribution."

Lowe and Gilbertson (4) note the similarity of P. durescens and P. palustris but state that the former occurs on angiosperms and the latter on conifers.

A study of P. palustris cultures in the Beltsville Forest Disease Laboratory was made in comparison with cultures of other fungi having similar characteristics. Among them were cultures in our collection under the name P. durescens, which had been so identified by comparison with an isolate (FP 48293-S)[2] from a basidiocarp determined by L. O. Overholts as P. durescens and on the basis of their occurrence on hardwoods. A collection of Polyporus spp. cultures from living peach trees in South Carolina sent to the laboratory for determination by Petersen (12) in 1959 were also indistinguishable from those of P. palustris. Examination of basidiocarps from which cultures had been obtained showed spores and basidia identical in size and shape with those of P. palustris.

Because dicaryotic cultures from basidiocarps identified as P. palustris could not be distinguished from those identified as P. durescens except by determination of the host and distribution, it seemed of value to apply the technique of pairing single spore cultures. This follows the method used by many authors, Mounce and Macrae (5, 6), Nobles (9), McKeen (8), McKay (7), Weresub and Gibson (13), and Denyer (2), as an aid in determining confused species. As pointed out in these studies, two basidiocarps in question may be considered as belonging to the same species if monosporous mycelia from the two are interfertile when grown together, whereas the failure to form a compatible combination strongly indicates the presence of two species. In such pairings of heterothallic species interfertility is demonstrated by the presence of clamp connections in the hyphae of the paired cultures and lack of fertility by the absence of clamp connections.

ISOLATIONS OF MONOSPOROUS CULTURES

Monosporous cultures were obtained by inducing the dicaryotic cultures in our collection to produce fruiting bodies. Single spores were isolated by methods described in a previous study (7).

Nine cultures named P. palustris from coniferous hosts together with ten from hardwoods considered to be P. durescens and nineteen similar cultures isolated from living peach trees by Petersen (12) were used in this interfertility study (Table 1). In addition to these, monocaryotic cultures of several Polyporus spp. and one from Brazil (Brazil-15) of questionable identity but with similar cultural characteristics were included. Five cultures from basidiocarps authoritatively determined as P. spraguei were also checked in this study since the cultures are quite similar to those of P. palustris and because the identity of the basidiocarps of P. durescens and P. spraguei had been confused before Overholts (10) described P. durescens as a separate species.

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[2] It is realized that this specimen may have been incorrectly identified, in which case we have no authentic cultures representative of P. durescens.

Table 1. Cultures used in interfertility studies.

Culture No.	Host	Location	Collector	Collection Date
Polyporus palustris				
Cultures from conifers:				
MS-48	On *Taxodium distichum* (L.) Rich. tanning drum; brown rot	Racine, Wisconsin	T. C. Scheffer	3-30-56
Mad. 4787	On *Pseudosuga menziesii* (Mirb.) Franco, flight deck planking		Culture received from C. A. Richards	9-1-49
FP* 57010 (Nobles #10618 (9))	On southern yellow pine	Hammond, La.	R. W. Davidson	6-13-32
FP 94152-R	On dead pine tree trunk causing brown rot	South Carolina	George H. Hepting	10-1941
FP 97400-S	On pine stump	Athens, Georgia	W. A. Campbell, J. L. Lowe	8-1950
FP 103991-R	On pine boards in dye mill roof	Anderson, S. C.	W. A. Campbell	10-27-53
FP 105322-S	On longleaf pine	Alma, Georgia	W. A. Campbell George H. Hepting	2-18-50
FP 105323-S	*Pinus elliotii* Engelm.	Homerville, Ga.	W. A. Campbell George H. Hepting	2-14-50
FP 105323-R	*Pinus elliotii* Engelm.	" "	Lee Hutchins, G. H. Hepting, W. A. Campbell	2-14-50
P-13a-R	*Pinus densiflora* Sieb. &	Nobeoka, Miyazaki, Japan	K. Aoshima	1-1948
Cultures from hardwood				
Roth 8	On decayed mahogany plywood hull of ship	Norfolk, Va. (Shipyard)	Elmer Roth	1-2-52
Roth 10	" " " "	" "	" "	" "
H-455-R	Ash(?) grating on bridge of warship	Charleston, S. C.	Carl H. Hartley	3-1949
4828	On yellow poplar trolley coach door		Culture received from C. A. Richards	6-20-50
5096-48	Red oak railroad tie	Seaboard Railroad S.E. Coast, U. S.	Forest Products Lab., Madison, Wis.	3-54
5096-52	White oak railroad tie	" " "	" " "	" "
FP 48293-S	Red maple log with brown rot	Washington, D. C.	J. A. Stevenson, det. by L. O. Overholts	7-1946
FP 71096	Sycamore	Mount Vernon, Va.	G. F. Gravatt W. A. Campbell	6-1936
FP 72012-Sp	Oak stump	Washington, D. C.	K. D. Doak	8-12-38
LCF 585	*Araucaria araucana* K. Koch timber	Neuguén, Argentina	Celina Iaconis	2-19-45
FP 59162-R	Apple stump		J. S. Cooley	3-1935
FP 104994-R	*Melia azedarach* L.	Athens, Ga.	R. W. Davidson W. A. Campbell	5-16-58
FP 103951-Sp	Fallen oak tree partly submerged in salt water	Camp Roosevelt, Calvert Co., Md.	H. H. McKay	6-30-53
16 cultures from peach (numbered in Table II)	Pruning wounds and stumps of peach trees	South Carolina	D. H. Peterson	June & July 1958 & 1959
Polyporus sp.				
Brazil-15		Sao Paulo, Brazil	Culture received from A. R. Teixeira	9-6-51
FP 50336-R	On ash, causing a crumbling dry rot	Oak Grove, La.		8-13-31
FP 105261-R	Tree in desert	Near Jachitan, Oaxaca, Mexico	Mason Hale, Jr. T. R. Soderstrom	3-1960
3 cultures from peach (numbered in Table II)	Pruning wounds	South Carolina	D. H. Peterson	7-1959
Polyporus spraguei				
FP 47476-S	White oak, heartwood railroad tie with brown cubical rot	Devil's Hole, W.Va.	D. Chapman, det. L. O. Overholts	9-3-28
FP 24658-S (ATCC 9381)			L. O. Overholts	
FP 59068-S	Chestnut telephone pole	Clarendon, Va.	R. W. Davidson, det. J. L. Lowe	1934
FP 103257-S	*Persea borbonia* (L.) Spreng. tree base with brown cubical rot	Baker Co., Fla., near Olustee	coll. & det. by A. S. Rhoads	9-25-52
FP 103258-S	*Gordonia lasianthas* Ellis, with friable brown heartrot	Osceola Natl. Forest Olustee, Fla.	coll. & det. by A. S. Rhoads	9-25-52

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* Prefix FP - Indicates numbers in the permanent series of the Forest Disease Laboratory
Suffix S - Culture from basidiocarp tissue
Suffix R - Culture from rot
Suffix Sp - Culture from spores

Table 2. Results of pairing monosporous mycelia.

	Polyporus palustris																																							*Polyporus* sp.						*Polyporus spraguei*				
	on conifer										on hardwood													on peach trees																on peach			on other hardwood			on hardwood				
	MS-48	Mad. 4787	FP 57010	FP 94152-R	FP 97400-S	FP 103991-R	FP 105322-S	FP 105323-S	FP 105323-R	PA-13a-R	Roth 8	Roth 10	H455-R	4828	5096-48	5096-52	FP 48293-S	FP 71096	FP 72012-Sp	LCF 585	FP 59162-R	FP 104994-R	FP 103951-Sp	FP 105428-R	FP 105424-R	FP 105391-S	FP 105390-S	FP 105393-S	FP 105394-Sp	FP 105395-S	FP 105395-Sp	FP 105396-S	FP 105397-S	FP 105398-S	FP 105399-S	FP 105400-S	FP 105401-S	FP 105402-S	FP 105403-S	FP 105421-S	FP 105413-S	FP 105407-S	Brazil-15	FP 50336-R	FP 105261-R	FP 47476-S	FP 24658-S	FP 59068-S	FP 103257-S	FP 103258-S
MS-48		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
Mad. 4787	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 57010	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+					+	+	+						0	0	0	0	0	0	0	0	0	0	0
FP 94152-R	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+					+	+	+						0	0	0	0	0	0	0	0	0	0	0
FP 97400-S	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 103991-R	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 105322-S	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 105323-S	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 105323-R	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+											+	+	+	0	0	0	0	0	0	0	0	0	0	0
P-13a-R	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+	+		+	+												0	0	0	0	0	0					
Roth 8	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					+	+	+	0	0	0	0	0	0	0	0	0	0	0
Roth 10	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+					+	+		0	0	0	0	0	0					
H455-R	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+																				0	0	0	0	0						
4828	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+				+			+													0	0	0	0	0						
5096-48	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+				+			+													0		0	0							
5096-52	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+				+			+															0	0							
FP 48293-S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 71096	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+									+	+	0	0	0	0	0	0					
FP 72012-Sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+					+	+	0	0	0	0	0	0	0	0	0	0	0
LCF 585	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+									+	+	+									0	0			0					
FP 59162-R	+	+	+	+	+	+	+	+	+		+	+					+	+	+	+		+	+	+	+	+	+	+	+	+										0	0	0	0	0	0	0	0	0	0	0
FP 104994-R	+	+	+	+	+	+	+	+	+		+	+					+	+	+		+		+	+	+	+	+	+	+	+										0	0	0	0	0	0	0	0	0	0	0
FP 103951-Sp	+	+	+	+	+	+	+	+	+		+	+					+	+	+		+	+		+	+	+	+	+	+	+										0	0	0	0	0	0	0	0	0	0	0
FP 105428-R (P89W)*	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 105424-R (P183W)	+	+			+	+	+	+	+	+	+	+					+	+	+		+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 105391-S (P249FB)					+	+	+	+	+		+						+		+		+	+	+	+	+		+	+	+	+	+					+				0	0	0	0	0	0	0	0	0	0	0
FP 105390-S (P251FB)	+	+	+	+	+	+	+	+		+	+	+		+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+					+				0	0	0	0	0	0	0	0	0	0	0
FP 105393-S (S289FB)	+	+			+	+	+	+		+	+	+					+	+	+		+	+	+	+	+	+	+		+	+	+					+				0	0	0	0	0	0	0	0	0	0	0
FP 105394-Sp (P370Sp)	+	+			+	+	+	+			+	+					+	+	+		+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
FP 105395-S (P323FB)	+	+				+	+	+			+	+					+		+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	0	0	0								
FP 105395-Sp (P323Sp)	+	+						+			+	+					+		+	+				+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	0	0	0								
FP 105396-Sp (S388FB)	+	+	+	+				+			+	+					+		+	+				+	+				+	+	+		+					+	+	0	0	0								
FP 105397-S (P390FB)	+	+	+	+	+	+	+	+									+		+					+	+				+	+	+	+						+	+	0	0	0								
FP 105398-S (P391FB)	+	+	+	+	+	+	+	+									+							+	+				+	+	+							+	+	0	0	0								
FP 105399-S (P392FB)	+	+			+	+	+	+									+							+	+				+	+	+							+	+	0	0	0				0	0	0	0	0
FP 105400-S (P395FB)	+	+			+	+	+	+									+							+	+	+	+	+	+	+	+						+	+	+	0	0	0								
FP 105401-S (P399FB)	+	+			+	+	+	+	+		+	+					+							+	+				+	+	+					+		+	+	0	0	0								
FP 105402-S (S402FB)	+	+			+	+	+	+	+		+	+					+	+	+					+	+				+	+	+	+	+	+	+	+	+		+	0	0	0								
FP 105403-S (S403FB)	+	+			+	+	+	+	+		+						+	+	+					+	+				+	+	+	+	+	+	+	+	+	+		0	0									
FP 105421-S (P362FB)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		+	+	+	+	+	0	0	0	0	0
FP 105413-S (P398FB)	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+		+	+	+	+	0	0	0	0	0
FP 105407-S (P400FB)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		+	+		+	+	+	0	0	0	0	0
Brazil-15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0											+	+	+		+	+	0	0	0	0	0
FP 50336-R	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0		0	0	0	0	0	0	0	0	0											+	+	+	+		+	0	0	0	0	0
FP 105261-R	0	0	0	0	0	0	0	0	0	0	0	0					0	0	0	0	0	0	0	0	0	0	0	0	0											+	+	+	+	+		0	0	0	0	0
FP 47476-S	0	0	0	0	0	0	0	0	0		0						0		0		0	0	0	0	0	0	0	0	0						0					0	0	0	0	0	0		+	+	+	+
FP 24658-S	0	0	0	0	0	0	0	0	0		0						0		0		0	0	0	0	0	0	0	0	0						0					0	0	0	0	0	0	+		+	+	+
FP 59068-S	0	0	0	0	0	0	0	0	0		0						0		0		0	0	0	0	0	0	0	0	0						0					0	0	0	0	0	0	+	+		+	+
FP 103257-S	0	0	0	0	0	0	0	0	0		0						0		0		0	0	0	0	0	0	0	0	0						0					0	0	0	0	0	0	+	+	+		+
FP 103258-S	0	0	0	0	0	0	0	0	0		0						0		0		0	0	0	0	0	0	0	0	0						0					0	0	0	0	0	0	+	+	+	+	

* The numbers in parentheses are those of D. H. Peterson.

INTERFERTILITY TESTS

Monosporous mycelia of the isolates in question were grown together in all possible combinations for 10 to 14 days, then examined for the presence of clamp connections. The results are shown in Table 2, where the + sign indicates the presence of clamp connections and compatibility between the pair involved and the zero sign indicates the lack of clamps and incompatibility.

Polyporus palustris was demonstrated to be heterothallic and bipolar by Nobles (9). In the present study, confirmation of bipolarity was made by pairing 20 monosporous cultures originating from a single basidiocarp of FP 94152-R produced in culture. When paired in all possible combinations the monosporous cultures segregated into two polarity groups, A and B.

DISCUSSION

The evidence shown in Table 2 demonstrates complete compatibility between monosporous cultures from basidiocarps of *P. palustris* from conifers and those from hardwood species known in our collection as *P. durescens*, as well as 16 of those from living peach trees in South Carolina (12). This and the similarity of basidiocarp characters and spore size demonstrates that *P. palustris* may occur on hardwood as well as conifer species and is widely distributed. Some basidiocarps from hardwoods may differ so greatly from the usual concept of *P. palustris* on conifers as to defy identification except by use of interfertility studies of monocaryotic cultures. Such was the case with some of the basidiocarps from living peach trees collected by Petersen (12). *P. spraguei* failed in all cases to pair with *P. palustris*, confirming its identity as a separate species.

Three of the peach tree isolates were compatible with a culture from Brazil (Brazil-15), one from Mexico (FP 105261-R), and one from Louisiana (FP 50336-R). This species remains to be identified. Three of the undetermined *Polyporus* spp., FP 59162-R from apple, FP 104994 from Chinaberry tree, and 103951-Sp from oak, were shown to be *P. palustris* by the interfertility test.

Monosporous mycelia obtained from a polysporous culture, P-13a, collected by K. Aoshima in Japan from decay in *Pinus densiflora* Sieb. & Zucc. also paired readily with *P. palustris*. The basidiocarp from which this culture originated had been determined as *Tyromyces squalens* (Karst.) Imaz. or *Polyporus versiporus* Yasada but was thought to be *P. palustris* by Aoshima.

40, 634 As preparation of this paper is being completed, an account of a similar study made in India by Bakshi and Singh (1) on heart-rot and decay due to *P. palustris* points out similar conclusions. This study reports *P. palustris* as occurring in India on the hardwood species *Shorea robusta* and *Cassia nodosa*, as well as on the conifer *Pinus roxburghii*. Bakshi and Singh demonstrate *P. palustris* to be heterothallic and bipolar and by interfertility studies show isolates from hardwood and conifer to be compatible. This is in complete agreement with the results presented in the present study.

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UNDESCRIBED PLANT DISEASES AND NEW HOSTS RECORDED IN OREGON DURING 1960-1961

Robert S. Halliwell and Iain C. MacSwan[1]

During the period July 1960 to September 30, 1961, several new diseases and diseases not previously reported for Oregon were recorded. In addition, several fungi were observed on plants not previously reported as hosts. The determination that diseases are undescribed and hosts are unreported is based on the Index of Plant Diseases in the United States (3) and the Host Fungus Index for the Pacific Northwest (1, 2). Disease diagnosis and fungus identification were made by the staff of the Oregon State University Plant Clinic, a cooperative project of the Oregon State University Federal Cooperative Extension Service and the Department of Botany and Plant Pathology.

A. New Diseases
   1. Root and crown rot of Fuchsia cuttings caused by *Thielaviopsis* sp.[2]
   2. Bacterial leaf blight of African violet (*Saintpaulia ionantha*) caused by *Pseudomonas* sp.
   3. Bacterial leaf blight of gloxinia (*Senningia speciosa*) caused by *Pseudomonas* sp.

B. Diseases new to Oregon
   1. Rhododendron twig blight and stem canker caused by *Phomopsis* sp.[2]
   2. Leaf streak and stunt of Iris incited by tomato ring spot.
   3. *Gloeosporium* sp. stem canker on azalea.
   4. Stem canker of *Vinca major* incited by *Phoma* sp.
   5. Black pox of apple incited by *Helminthosporium papulosum* Berg[2] was found on the variety Delicious.
   6. Leaf blight of field-grown tomatoes incited by *Cladosporium* sp.[2]
   7. Black scurf of garlic incited by *Helminthosporium allii* Campanile[2] was observed numerous times.
   8. Mottle and distortion of spinach leaves incited by cucumber mosaic virus[2].
   9. Fusarium wilt of parsley (*Petroselinum crispum*) incited by *F. oxysporum* Schlecht. *f. apii* (R. Nelson & Sherb.) Snyd. & Hans.[2]

C. New Hosts
   1. *Cytospora* sp.[2] - twig blight of Japanese andromeda (*Pieris japonica*) was encountered in two separate incidents.
   2. *Phomopsis* sp.[2] - twig and stem blight of Oregon grape (*Mahonia aquifolium*).
   3. *Heterosporium* sp.[2] - tip blight of arborvitae (*Thuja*).
   4. *Phomopsis* sp.[2] - necrotic leaf spot of *Torreyae* and *Cunninghamia*.
   5. *Entomosporium* sp.[2] - leaf spot of *Raphiolepis indica*.

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[2] Cultures are maintained in the Plant Clinic Culture Collection.

CONTROL OF POSTHARVEST DECAYS OF PEACHES[1]

R. A. Cappellini and A. W. Stretch

Summary

A formulation of 2,6-dichloro-4-nitroaniline gave excellent and consistent control of Rhizopus rot of peaches at 1000 ppm. Treatment of peaches 12 hours after inoculation and suitable incubation completely suppressed Rhizopus rot for 3 days at 75° to 80° F. Control of brown rot was not as effective, but it was noted that this material restricted the size of lesions produced by *Monilinia fructicola*. No appreciable absorption of this material through uninjured epidermis was detected.

[Scl

INTRODUCTION

The two primary diseases of harvested peaches are brown rot, caused by *Monilinia fructicola* (Wint.) Honey, and Rhizopus rot, caused by *Rhizopus nigricans* Ehr. Reports on postharvest chemical treatment of peaches for both of these rots have been published (2, 3, 4, 6, 7), but the most consistent and outstanding control has been obtained against *M. fructicola*. Successful commercial control of *R. nigricans* is still lacking. Infection by *R. nigricans* occurs chiefly through wounds in the epidermis and spreads relatively quickly through the fruit; serious consequence of this infection is the quick growth over and decay of adjacent fruit (nesting effect).

Tests conducted at the New Jersey station in 1960 and 1961 have shown that 2,6-dichloro-4-nitroaniline, applied as a short-time dip, will give effective and consistent control of Rhizopus rot. Some control of brown rot was obtained but the results are not as striking.

Material for the 1960 tests was obtained from the British manufacturer[2]. The width of the spectrum of activity of this material had not been fully established but in a series of *in vitro* tests it appeared to be almost specific to *Botrytis* sp. (1). Reports submitted indicated the excellent activity of this material against *Rhizopus* on peaches. Comparable data on sweet cherries from work begun in 1961 have been published by workers in California (5). The postharvest use of 2,6-dichloro-4-nitroaniline on peaches has not, as yet, been approved.

Our 1961 tests on peaches with Allisan and Botran have shown little or no difference in activity of either formulation against *Rhizopus*.

Tests reported in this paper were conducted to determine the effects of various concentrations of 2,6-dichloro-4-nitroaniline on Rhizopus and brown rot, the inoculation-treatment interval in which decay is suppressed, and the degree of absorption of material through the uninjured peach epidermis.

MATERIALS AND METHODS

In all tests, mixed varieties of sound, firm-ripe peaches were randomized and inoculated with spore suspensions (50-100,000/cc) of either *R. nigricans* or *M. fructicola*. Spores were harvested from 7-day cultures originally isolated from peaches and grown on potato-dextrose agar. To insure uniform infection with *R. nigricans*, a small area of the epidermis was removed and a drop of spore suspension placed in the wound. Inoculations with *M. fructicola* were made by spraying a spore suspension on one check of the fruit. A 50% wettable powder formulation of 2,6-dichloro-4-nitroaniline was used in all cases.

RESULTS AND DISCUSSION

Control of Rhizopus and Brown Rot: Peaches were inoculated as outlined, and after 2 to 3 hours' incubation were dipped for 1 to 2 minutes in 250, 500, 1000, and 2000 ppm chemical. After treatment they were stored in 4-quart baskets under conditions of high humidity at 75° to 80° F. Evaluations were made after 3 and 5 days' storage for Rhizopus rot, and 4 and 6 days for brown rot. Data were taken on 12 peaches in each of five replications in each of two tests.

Considerable reduction in Rhizopus rot was obtained with 1000 and 500 ppm chemical in

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[1] Paper of the Journal series, New Jersey Agricultural Experiment Station, Rutgers the State University, Department of Plant Pathology, New Brunswick.

[2] Boots Pure Drug Co., Ltd., Nottingham, England who have sold it under the name Allisan. The Upjohn Co., Kalamazoo, Michigan distributes it in the U.S., first experimentally as U-2069 and now commercially as Botran.

Table 1. Effects of various concentrations of 2,6-dichloro-4-nitroaniline on the postharvest control of Rhizopus rot in peaches.

Concentration (ppm)	Average % infected fruit after: 3 days, inoculated	5 days, inoculated	3 days, uninoculated	5 days, uninoculated
0	81	87	13	33
250	68	70	4	15
500	55	62	0	9
1000	12	13	0	3
2000	5	6	0	2

Table 2. Effects of various concentrations of 2,6-dichloro-4-nitroaniline on postharvest control of brown rot in artificially inoculated peaches.

Concentration (ppm)	Average % infected fruit after: 4 days	6 days
0	56	65
250	40	71
500	49	76
1000	28	48
2000	22	30

Table 3. Effects of 1000 ppm 2,6-dichloro-4-nitroaniline on the control of Rhizopus rot after varying lengths of time between inoculation and treatment.

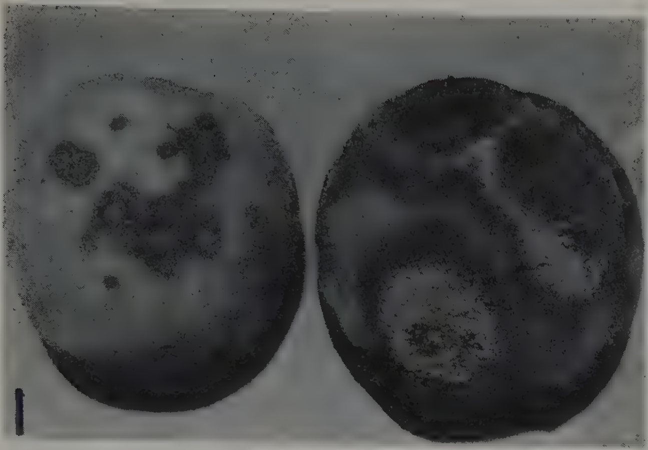
Inoculation-treatment interval (hours)	Peaches/test	% infected peaches: Trt.	% infected peaches: Cks.	% decay[a]: Trt.	% decay[a]: Cks.
0	20/2	0	80	0	90
3	30/3	0	85	0	85
6	30/3	0	85	0	85
9	70/5	0	93	0	90
12	70/3	0	89	0	95
15	30/3	32	90	31	90
18	30/3	68	85	40	90
21	30/3	73	90	74	85
24	30/3	76	85	77	95

[a] Approximate % decay was calculated by obtaining the radius of decay/diameter of peach x 100.

inoculated and uninoculated lots, respectively (Table 1). Throughout the test period "nesting" was prevented by the 1000 and 2000 ppm treatments.

In the brown rot tests, the data indicate that control by this material is inadequate when the inoculum potential of M. fructicola is high (Table 2). The size of the lesions produced by this organism, however, is considerably restricted by 1000 and 2000 ppm 2,6-dichloro-4-nitroaniline (Fig. 1). At 250 and 500 ppm, lesions intermediate in size between those at the higher concentrations and the controls were observed. This material may give commercially acceptable control of M. fructicola under more realistic and natural conditions. It is noteworthy that the appearance of the restricted lesions resulting from this material is not unlike the symptoms produced by Glomerella cingulata (Ston.) Spauld. & Von Schrenk.

Post Inoculation Treatments: These tests were designed to determine the inoculation-treatment interval in which Rhizopus rot was completely suppressed for 3 days at 75° to 80° F. Test peaches were dipped for 1 minute in 1000 ppm chemical at 0, 3, 6, 9, 12, 15, 18, 21, and 24 hours after inoculation. Data were obtained on the number of peaches infected and the radii of the lesions. Each replicate consisted of 10 peaches. The results (Table 3) show that complete control can be obtained for 3 days by treatment up to 12 hours after inoculation. Treatment 15 hours after inoculation permitted decay in 32% of the fruit. Little control was obtained with treatment 18 to 24 hours after inoculation. The extent of decay within the peach in the treatments 15 and 18 hours after inoculation was approximately half that at 21 and 24 hours (Table 3 and Fig. 2).



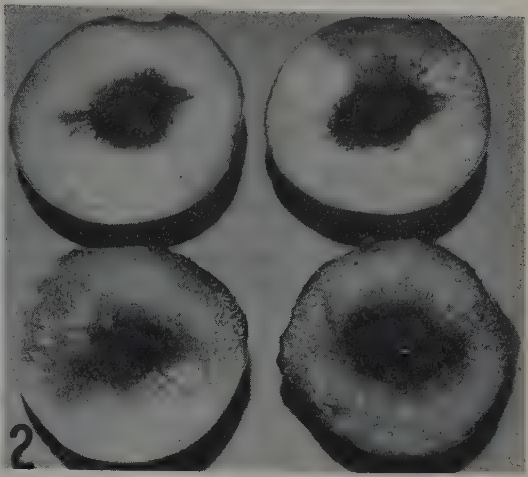


FIGURE 1. Effects of 2,6-dichloro-4-nitroaniline on brown rot after 6 days' storage at 75° to 80° F. Left -- treated with 1000 ppm. Right -- untreated.

FIGURE 2. Extent of Rhizopus rot after 3 days' storage at 75° to 80° F in peaches dipped for 1 minute in 1000 ppm 2,6-dichloro-4-nitroaniline after various inoculation-treatment intervals. Upper left -- treatment up to 12 hours after inoculation. Upper right -- 18 hours after inoculation. Lower left -- 24 hours after inoculation. Lower right -- untreated.

Chemical Absorption Through Uninjured Peach Epidermis: To determine whether 2,6-dichloro-4-nitroaniline is absorbed through the epidermis, peaches were dipped in 1000 ppm for 1/2, 1, 2, 5, 10, and 20 minutes at 75° F and immediately air-dried. The test peaches were then inoculated by placing a very small drop of inoculum (500,000 Rhizopus spores/cc) 5 mm into 10 peaches in each of two replications through a puncture 1 mm in diameter. The objective was to assess absorption by following internal decay to the periphery of the fruit at points away from the site of inoculation. The failure of Rhizopus to reach an area near the periphery was to be taken as evidence of absorption of the material through the fruit.

In all cases decay progressed to the epidermis after 3 days' storage at 75° to 80° F. Rhizopus mycelium was observed microscopically in the tissue immediately under the epidermis. This indicates that there is little or no absorption of 2,6-dichloro-4-nitroaniline by peaches.

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PRELIMINARY LIST OF PATHOGENIC FUNGI OF FOREIGN FOREST TREES PLANTED IN TOKYO UNIVERSITY FOREST, HOKKAIDO, JAPAN

Haruyoshi Saho[1]

INTRODUCTION

Since 1916, many foreign forest trees have been planted in Tokyo University Forest in Hokkaido. Currently, large-scale test plantations of foreign forest trees are progressing in accordance with the plans of Professor N. Takahashi, Director of the Forest.

The writer is responsible for investigations of the diseases of trees planted in the University Forest. This is a preliminary report of the pathogens of foreign forest trees observed from 1958 to 1961. At a later date a final report of all known pathogenic fungi of foreign forest trees in the Forest will be prepared.

PATHOGENIC FUNGI

1) Armillaria mellea (Fr.) Quél. Root rot

Although according to the soil conditions, A. mellea attacks principally the trees in young plantations that are planted improperly, we have observed infections also on trees injured in various ways; for example, damaged by hares, voles, insects, and needle-rusts. Therefore, the forester must not only plant his trees carefully but he must protect them from injuries to prevent infections.

Hosts: Larix decidua, L. gmelini (L. dahurica), L. gmelini var. koreana, L. gmelini var. olgensis, Picea abies, Pinus banksiana, P. koraiensis, P. strobus, P. sylvestris.

2) Botrytis cinerea Pers. ex Fr. Gray mold of seedling

Hosts: Picea abies, Pinus griffithii, P. cembra.

3) Coleosporium cacaliae Otth. Soft pine needle rust. Heteroecious. Alternate host: Cacalia hastata subsp. orientalis

In Europe, C. cacaliae is pathogenic on two-needle pines, but the writer's sporidia inoculation experiments show that C. cacaliae in Hokkaido is pathogenic on five-needle pines. C. cacaliae in Hokkaido may be a different species from the one in Europe.

Hosts: Pinus koraiensis, P. strobus.

4) Coleosporium eupatorii Arth. Soft pine needle rust. Heteroecious. Alternate host: Eupatorium chinese subsp. sachalinense

Widespread. This disease is serious in young plantations. The current height growth of diseased trees is 30 to 40% less than that of healthy trees. By the writer's sporidia inoculation experiments, many five-needle pines are susceptible.

Hosts: Pinus koraiensis, P. strobus, P. monticola.

5) Coleosporium petasitis Lév. var. yamabense Saho Soft pine needle rust. Heteroecious. Alternate host: Petasites japonicus subsp. giganteus

In Europe, C. petasitis is pathogenic on Pinus sylvestris but the writer's sporidia and aeciospore inoculation experiments show that the rust on P. japonicus subsp. giganteus is pathogenic on five-needle pines.

Hosts: Pinus koraiensis, P. strobus.

6) Coleosporium phellodendri Kom. Hard pine needle rust. Heteroecious. Alternate host: Phellodendron sachalinense

Widespread. By the writer's inoculation experiments, it was 2 years between sporidia inoculation and peridermium formation.

Hosts: Pinus banksiana, P. sylvestris.

7) Coleosporium senecionis Fr. (?) Soft pine needle rust. Heteroecious. Alternate host: Senecio cannabifolius

In Europe and America, this fungus is pathogenic on two-needle pines but the writer's sporidia inoculation experiments show that the rust on S. cannabifolius is pathogenic on five-needle pines. This fungus may be a new variety.

Hosts: Pinus koraiensis, P. strobus.

8) Cronartium quercuum Miyabe Hard pine gall rust. Heteroecious. Alternate host: Quercus crispula

Causes galls of twigs and branches of hard pine.

Host: Pinus sylvestris.

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9) *Cytospora chrysosperma* (Pers.) Fr. Poplar bark canker
Causes bark canker of *Populus* spp. Especially, "Peace" was the most susceptible.
Hosts: *Populus* spp.

10) *Dasyscypha* spp. Conifer bark canker
Widespread. Causes bark canker of weakened trees.
Hosts: *Larix occidentalis*, *Pinus strobus*.

11) *Helicobasidium mompa* Tanaka
Found on the surface of the bark at the base of trees. Occasionally, diseased trees had been previously attacked by *Armillaria mellea*.
Hosts: *Pinus strobus*, *Larix decidua*.

12) *Lophodermium pinastri* Chev. Pine leaf cast
Hosts: *Pinus contorta* var. *murrayana*, *P. ponderosa*, *P. rigida*, *P. resinosa*, *P. strobus*, *P. sylvestris*.

13) *Melampsora abietis-populi* S. Imai Fir-poplar rust. Heteroecious
Very rare.
Hosts: *Abies concolor*, *Populus* spp.

14) *Melampsora larici-populina* Kleb. Larch-poplar rust. Heteroecious
The most common poplar leaf rust.
Hosts: *Larix decidua*, *L. gmelini* (*L. dahurica*), *L. gmelini* var. *koreana*, *L. gmelini* var. *olgensis*, *L. gmelini* var. *principis-rupprechtii*, *L. laricina*, *L. occidentalis*, *L. sibirica*, *Populus* spp.

15) *Mycosphaerella larici-leptolepis* I. Ito, Sato et Ota Larch needle cast
One of the three serious diseases of larch in Japan. The other two diseases are Armillaria root rot and Physalospora twig blight. The foreign larch species are more susceptible than Japanese larch according to the writer's investigations.
Hosts: *Larix decidua*, *L. gmelini* (*L. dahurica*), *L. gmelini* var. *koreana*, *L. gmelini* var. *olgensis*, *L. gmelini* var. *principis-rupprechtii*, *L. laricina*, *L. occidentalis*, *L. sibirica*.

16) *Melampsoridium alni* Diet., *Melampsoridium hiratsukanum* S. Ito Larch-alder rust. Heteroecious
Alnus incana is the most susceptible according to the writer's investigations.
Hosts: *Alnus incana*, *A. glutinosa*, *A. rhombifolia*, *A. rubra*, *A. sinuata*, *A. tenuifolia*, *Larix decidua*, *L. gmelini* var. *koreana*.

17) *Melampsoridium betulinum* Kleb. Larch-birch rust. Heteroecious
No uredosorus on the leaves of *Betula lenta*.
Hosts: *Betula nigra*, *B. papyrifera*, *B. pendula*, *B. populifolia*, *B. pubescens*, *B. verrucosa*, *Larix decidua*, *L. gmelini* var. *koreana*.

18) *Phomopsis occulta* Trav. Conifer stem canker
Hosts: *Pinus strobus*, *P. sylvestris*.

19) *Physalospora laricina* Sawada Larch twig blight
Widespread in Hokkaido and Northern-honshū. The most dangerous disease of larch in Japan. All species of larch are affected.
Hosts: *Larix gmelini* (*L. dahurica*), *L. gmelini* var. *koreana*.

20) *Pucciniastrum tiliae* Miyabe Fir-linden rust. Heteroecious. Alternate host: *Tilia maximowicziana*
Hosts: *Abies concolor*, *Tilia americana*.

21) *Rhizoctonia solani* Kuehn Root rot
Causes damping-off of young coniferous seedlings.
Hosts: *Pinus monticola*, *P. sylvestris*.

The following are the fungi found on dead twigs, branches, and stumps.

22) *Coriolus hirsutus* (Wulf. ex Fr.) Quél.
Host: *Catalpa speciosa*.

23) *Fomotopsis insularis* Imaz.
Host: *Picea abies*.

24) *Gloeophyllum sepiarium* (Wulf. ex Fr.) Karst.
Host: *Picea abies*.

25) *Hirscioporus fusco-violaceus* Donk
Host: *Picea abies*.

26) *Lenzites betulina* Fr.
Host: *Picea abies*.

27) *Stereum sanguinolentum* (A. ex S.) Fr.
Host: *Pinus strobus*.

FOREST PATHOLOGY LABORATORY, TOKYO UNIVERSITY FOREST,
HOKKAIDO, JAPAN

SUSCEPTIBILITY OF WILDFIRE-RESISTANT TOBACCO VARIETIES TO CERTAIN STRAINS OF PSEUDOMONAS TABACI AND P. ANGULATA[1]

W. D. Valleau, C. C. Litton, and E. M. Johnson

The development of wildfire-resistant tobaccos using the resistance factor from Nicotiana longiflora gave promise of solving the wildfire (Pseudomonas tabaci (Wolf & Foster) F. L. Stevens) and angular leaf spot (P. angulata (Fromme & Murray) Holland) problems completely. But the work of Diachun and Troutman (3) proved that N. longiflora, from which resistance to wildfire was obtained, was not immune as had been claimed (1, 2) but only highly resistant, and the same is true for the resistant tobaccos carrying this factor. Bacteria multiply in leaves of both but at a slower rate than in leaves of susceptible plants. Diachun and Troutman prophesied that "Inasmuch as P. tabaci can multiply in leaf tissue of resistant plants, the possibility exists that, in time, strains of the organism that could cause symptoms in plants now resistant might evolve." More recently Heggestad, et al. (4) have been more conservative in their claims for the degree of resistance carried in Burley 21, recognizing that wildfire occasionally develops "either from the development of new strains of bacteria able to attack this or other similarly resistant varieties or from the appearance of some plants which have lost the genes for resistance."

In the breeding work at the Kentucky Agricultural Experiment Station, the N. longiflora factor has been introduced into both burley and dark tobacco lines in the hope that wildfire and angular leaf spot of burley would be eliminated and the blackfire disease of the dark-fired and air-cured tobacco, which seems to be caused by either or both organisms (6), would be brought under control. For purposes of selection, plants of the various breeding lines are sprayed in the plant bed and in the field with a 1:1000 or 1:2000 broth culture of the wildfire organism in water. In 1959 one of four wildfire strains carried in living leaves of Ky 16 tobacco since 1956 was selected at random and used in the field. The four strains were thought to be non-virulent on Burley 21. We were very much surprised to find that Burley 21 and all of our supposedly resistant breeding lines were highly susceptible to this strain (5). Later a graduate student (J. H. Smiley) isolated a similar strain after eight passages through Ky 16. Following some studies by him on these virulent strains both strains lost virulence and we have failed to recover it since.

During the spring of 1961 all specimens of angular leaf spot and wildfire sent to the laboratory for diagnosis were tested on Burley 21 to determine the prevalence of virulent strains of both organisms. Inoculations were made by crushing a small bit of affected tissue in about 20 cc of water and water soaking a leaf with this material with a syringe while the stomata were open. The results were about as follows: All collections diagnosed as angular leaf spot caused infection on Burley 21. A majority of the collections diagnosed as wildfire caused typical wildfire lesions on Burley 21. But when these wildfire strains were cultured in broth and diluted to 1:1500, they failed to cause any symptoms other than a faint chlorotic line where the tissue was water soaked with the dilution. Other wildfire collections caused no symptoms, other than the faint chlorotic line on Burley 21 when spots were crushed in water and used as inoculum.

While this work was in progress, a bed of Burley 21, 100 yards long, was found to be infected with wildfire from one end to the other. Records showed that the bed was sowed with certified Burley 21 seed, and subsequent observation of plants in the field indicated that they were typical Burley 21. The planting from this bed was examined July 7, 1961 after a rainy period. Wildfire was scattered over the field on lower leaves, and occasional higher leaves had large necrotic spots with abundance of bacteria in them. Near the bed area a few rows showed typical severe wildfire on all plants extending more than halfway up the plants. It looked like an ordinary susceptible variety. Collections were made from the bed and later from the field, and tested on susceptible (Ky 26) and resistant burley (Burley 21 and Exp. 12). Both collections proved to be highly virulent.

Dilution studies were made using a 24-hour broth culture incubated at 70° F. In one test dilutions were used ranging from 1:500 to 1:2500 at 500 intervals. Inoculations were made in two ways. Two leaves on a plant of Burley 21 were water soaked in five areas between large lateral veins and the five dilutions were applied with a glass spatula, each to a water-soaked area, applying the greatest dilution first and the lowest dilution last. Inoculations were repeated on three plants, or six leaves. The same dilutions were used to water soak streaks on the leaves. All dilutions applied in both ways caused typical broad chlorotic wildfire haloes, with some necrosis along the lines of inoculation. Further tests made the same way, using dilutions from 1:3000 to 1:8000 on somewhat older leaves, produced a small number of discrete wildfire spots at each dilution when the inoculum was applied with a spatula, but no symptoms whatever when the dilutions were used to water soak the leaves. The reason for this difference is not clear but earlier symptoms appeared in the 500-2500 dilution series where the inoculum was applied with a spatula.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

In view of the fact that burley varieties carrying the resistance factor from N. longiflora are highly susceptible to this field strain of the organism, it was of interest to know how N. longiflora itself would react. Inoculations were made by water soaking streaks on N. longiflora leaves and inoculating by drawing a drop of a 24-hour broth culture of W.F. 35, a strain that causes wildfire on susceptible plants only, and of W.F. 49 the highly virulent strain from Burley 21. On N. longiflora W. F. 35 caused an extremely faint chlorotic line hardly noticeable, while W. F. 49 caused a solid necrotic streak about 2 mm wide and a halo about 1 cm across, typical of wildfire. The same results were obtained when a half-leaf was inoculated with one culture and the other half with the other. A microscopic examination of the necrotic tissue proved that the bacteria had multiplied abundantly.

During the past several years we have had reports of Burley 21 being affected by angular leaf spot and have personally investigated some of these cases. There seems to be no doubt that Burley 21 is often severely damaged by angular leaf spot at any stage of its development. In 1961 many plantings of Burley 21 were seen that were heavily infected with angular leaf spot. In our breeding plots some of the wildfire resistant lines were heavily infected naturally with angular leaf spot. It is possible that Burley 21 and other resistant varieties may be resistant to certain strains of P. angulata, but it is certain that strains are common to which Burley 21 shows no appreciable resistance.

DISCUSSION

It is evident from these results that in general the common strains of P. angulata are more virulent than the common strains of P. tabaci on resistant varieties because Burley 21 in the bed and field is commonly heavily infected with P. angulata, while fields of Burley 21 are rarely seen heavily infected with P. tabaci. It is also evident that there is variation in the virulence of strains of P. tabaci as some collections cause no infection on Burley 21 when spots are crushed in water and used as inoculum, others cause infection with this relatively high concentration, but not when diluted, while culture W. F. 49 causes infection at high dilutions and causes heavy field infection on Burley 21 and typical wildfire on N. longiflora.

It is probable that the degree of resistance found in Burley 21 will continue to be useful in wildfire control for many years, but of little value in control of angular leaf spot. In view of the fact that wildfire was found throughout a bed 300 feet long that had received drainage water from a bluegrass field, it appears probable that the virulent strain is well established on the roots of pasture plants in this bluegrass field. Our studies on the wildfire organism (6) indicate that primary infections in tobacco beds are made up of mixtures of strains with different colony morphology (rough, smooth, and so forth) so it is not beyond reason that virulent strains of the wildfire organism may be widely scattered as mixtures. If so, it may be expected that with time there will be a gradual buildup of virulent strains in fields where a resistant variety is grown at frequent intervals.

The situation is more critical with dark tobaccos than with burley so far as the long-time viewpoint is concerned. Blackfire is apparently caused by either the wildfire or angular leaf spot organisms, or a mixture. Resistance to wildfire in Burley 21 does not appear to cause resistance to angular leaf spot and so the introduction of the longiflora factor into the dark-fired and dark air-cured tobaccos may be of little value in the control of blackfire. So far we have no critical field tests with wildfire resistant dark tobaccos and so we can only speculate.

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AN IRON DEFICIENCY OF PARA GRASS, BRACHIARIA MUTICA, AND GUINEA GRASS, PANICUM MAXIMUM, NOTED IN THE WESTERN REGION OF NIGERIA[1]

Warren N. Stoner[2]

Summary

A chlorosis of Para grass, Brachiaria mutica, and Guinea grass, Panicum maximum, was observed during the dry seasons of 1958 and 1959 at Ibadan, Nigeria. Trace element experiments showed the condition could be corrected by application of iron citrate solutions. A slightly alkaline soil pH and an increase of available phosphorus were correlated with occurrence of the chlorosis. A resumption of normal growth in the rainy season, plus a shift back to an acidic soil pH, and a decrease in available phosphorus indicate the chlorosis was due to a cyclic iron deficiency primarily contingent upon low soil moisture.

INTRODUCTION

Early in the month of November 1958, the attention of the author was drawn to an abnormal yellowing of the young tips of various plants of Para grass, Brachiaria mutica, growing in a small increase plot on Moor Plantation, Ibadan, Nigeria. Specimens from this plot were brought to the author by G. H. Ahlgren who was concerned that the abnormality might be caused by a virus infection. It was particularly important that the cause of the condition be ascertained since distribution of vegetative planting material from the plot was contemplated.

1958 FIELD OBSERVATIONS

An observation of the planting showed that about 5% of the plants were affected and that they were evenly distributed throughout the plot. Close examination of individual plants showed that not all of the tillers of any given plant were affected and the bottom leaves of many of the affected tillers appeared normal.

The main symptoms were observed in the tip leaf and the two or three next younger leaves below it. These leaves were evenly golden yellow to cream yellow in color with the youngest unfurled leaf often being almost white (Fig. 1B).

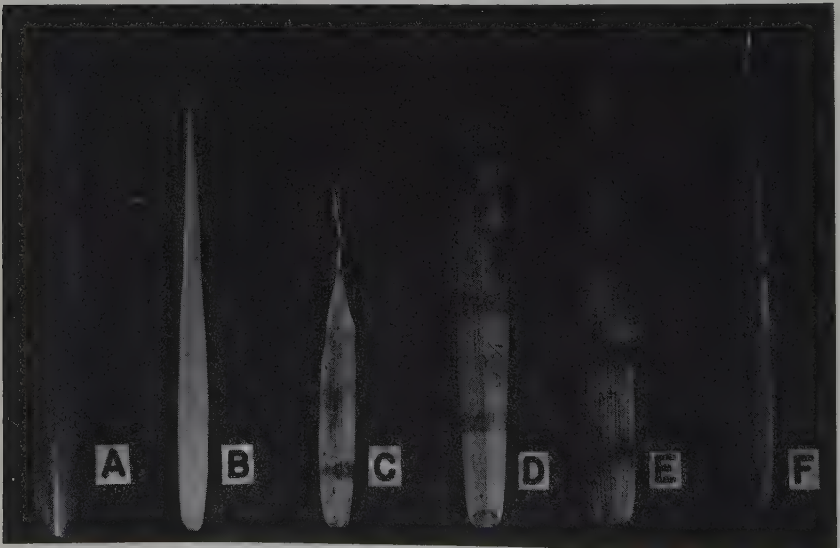

FIGURE 1. Leaves of Para grass, Brachiaria mutica. A -- normal. B -- extreme chlorosis observed in the field in tip leaves. C through F-- the gradient of response to solutions of ferric citrate at full, one-half, one-quarter, and one-eighth saturation, respectively. This picture was taken 4 days after application of the solutions. Note the necrosis developing in the darker areas of leaves C and D. Approx. 1/2 natural size.

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[1] This report is made on a portion of the investigations conducted under Project 62. 13. 050 of the International Cooperation Administration. It should be cross indexed under Project No. 9: Plant Virology, International Development Services Inc. I. C. A. contract "Mission to Western Nigeria."

[2] Formerly, Virologist, International Development Services Inc., Ministry of Agriculture and Natural Resources, I. D. S. Specialists Section, Moor Plantation, Ibadan, Nigeria. Now Entomologist, Northern Grain Insects Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Brookings, South Dakota.

Occasionally the fourth or fifth leaf down the stem from the tip would show faint to strong green lines confined to the veins. These veinal green streaks ran the entire length of the leaf without interruption. The even chlorosis, however, was by far the most striking and general symptom and seemed to be related to a rapid growth flush induced by heavy showers which occurred 10 days to 2 weeks prior to notice of the condition. An unseasonable dry spell had preceded these showers for about 30 days.

An examination of surrounding vegetation and nearby plots of other pasture grasses revealed similar but less severe symptoms in a plot of Guinea grass, Panicum maximum.

The above observations plus the strong resemblance to a similar condition induced in rice and grasses in Florida by iron deficiency, reported by Green and Stoner (2), indicated that the abnormality could be due to a shortage of this element. Moreover, iron deficiency has been reported as widespread by Lister and Thresh (4) in cocoa in the Western Region of Nigeria, and a deficiency of this element, of perhaps a temporary nature, in young oil palms has been noted in Nigeria by Bull (1) and again by Hewitt and Bull (3). These authors also indicate that iron deficiency may account for acute chlorosis symptoms they have observed in Pueria, Raphia palms, and in occasional plants of coffee.

FIELD EXPERIMENTS

Experiment 1: The range of trace element deficiencies listed as possibly occurring in Nigeria by Hewitt and Bull (3) indicated that it would be advisable to include a number of those elements in rough tests. This was done in an attempt to determine the cause of the chlorosis. On Friday, November 11, 1958 saturated solutions of zinc sulfate, manganese sulfate, ferrous sulfate, ferric citrate, and magnesium sulfate were prepared and applied in transverse strips across affected leaves of the Para grass with a small camel hair brush, and the tip of a tall Guinea grass shoot was dipped in the ferric citrate solution. Upon observation on the following Monday, the plants treated with ferric citrate were the only ones showing a reaction. Dark green bands were plainly evident on the Para grass leaves in the areas where the material had been applied. Moreover, the tip of Guinea grass dipped in the ferric citrate solution had also assumed a normal green color. Observations made over the next few days showed that the green area remained constant in size and sharply defined on the treated Para grass leaves. This, plus the fact that the chlorosis was present only in the young flush of growth induced by the rains, indicated immobility of the iron. Such immobility is well known as a characteristic behavior of iron in plants as indicated by Wallace (6) and others.

Experiment 2: On November 15, one day after the initial observation on the first experimental plants, a slight necrosis began to develop in the treated areas of the Para grass leaves. This necrosis may have been due to the high concentration of the solution used, therefore a similar series of applications were made on November 20 to test this possibility. In this second test, only ferric citrate was applied with camel hair brushes at concentrations of saturation, one-half saturation, one-fourth saturation, and one-eighth saturation. At the time of the application a representative soil sample was taken from the plot[3]. When the plants were examined 3 days later they had reacted the same as in the first trial. The amount of greening was relatively proportional to the strength of the solution used and slight necrosis was noted only on the plants treated with the saturated and half-saturated solutions (Fig. 1, C-F).

Experiment 3: On December 16 a third set of applications similar to those of the second experiment were made on affected Para grass, using ferric citrate at one, one-tenth, one-hundredth, and one-thousandth normal concentrations. Again, the degree of response was related to the concentration of the solution used. In this test however, the necrosis did not develop.

1959 FIELD OBSERVATIONS

At the beginning of the rainy season in April 1959, the Para grass resumed growth. The portions of the tillers that had been affected did not recover, but shoots from axillary buds on these tillers and new tillers on the affected plants were all normal in appearance. In May, about 6 weeks after new growth had started, a second soil sample was taken. This sample and the one taken in November 1958 were both submitted to the Western Region Soils Testing Laboratory for analysis. The soil sample taken in November 1958 had a pH of 7.1 or slightly alkaline, while the pH of the second sample taken in May 1959 was 6.6 or slightly acid. Phosphorus analysis indicated that available phosphorus was slightly higher in the November sample, taken when the condition was showing, than in the May sample, taken when normal growth had been resumed.

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[3] The author is indebted to Mr. A. Adegbola, Agricultural Officer of the Western Region Ministry of Agriculture, for taking this and subsequent soil samples and for making the second and third applications of the tests.

A short drouth period is usual in the region in July and August. By August 20, 1959, there had been very little rain for about a month and the symptoms described in the Para grass were beginning to reappear. This suggests that the problem may be cyclic in nature and indicates a possible correlation of the condition with the growth cycle as it is influenced by climatic factors, particularly rainfall.

DISCUSSION

Iron in a reduced form is known to be oxidized and consequently rendered relatively unavailable to a plant from the soil under conditions of alkalinity. Moreover, abundant available phosphorus also tends to tie up iron in relatively unavailable compounds as indicated by Scarseth and Salter (5) and Wallace (6). The characteristic immobility of the element in the plant means that for normal growth there must be a constant source of available iron in a reduced form. If, then, under normal conditions in a soil available iron was at the critical level, even small shifts in pH to a relatively higher value and an increase in soluble phosphorus could cause a significant drop in the availability of iron. If soil moisture should also be low at this time so that growth is retarded, the reduction of available iron might not be evident in the plant until such time as rapid growth is resumed. Under such conditions, a temporary shortage of iron could occur for a short time until the usual balance of the element was restored. The observations, soil analyses, and experiments completed indicate that just such conditions existed and caused the deficiency symptoms noted in this report.

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WATERMELON DISEASES ON THE CHICAGO MARKET 1960-1961

N. S. Luepschen and M. A. Smith[1]

This report is a brief account to enumerate the diseases of watermelons arriving on the Chicago market, their cause and relative importance. During the past two seasons observations were made of watermelon rail shipments to the Chicago Produce Terminal. Observations for 1960 were limited largely to instances of melon decay brought to our attention by the Western Weighing and Inspection Bureau personnel. In 1961, melon cars were inspected at approximately 10-day intervals to provide a sample survey of postharvest melon diseases. In most cases the pathogens were isolated to verify the symptom diagnoses.

ANTHRACNOSE

Colletotrichum lagenarium (Pass.) Ell. & Halst.

In 1960, in three cars of Charleston Gray melons from Missouri, an average of 10% of the melons were unsalable because of anthracnose lesions. In the 1961 survey, traces of this disease were seen throughout the summer in Charleston Gray carlots from the southern States. Anthracnose was also noticed on Black Diamond melons from Oklahoma.

BLOSSOM-END ROT

In July 1960, occasional melons in cars of Charleston Gray had dark brown, dry necrotic lesions on the blossom end. Blossom-end rot is considered to be of physiological origin (3). Isolations yielded such secondary organisms as *Fusarium* and *Alternaria*, and a *Phomopsis* sp. Watermelons and cucumbers were inoculated with the Phomopsis organism and a firm decay developed successfully. Alpha and beta spore measurements correspond to the range for *Phomopsis cucurbitae* McKeen (2). A *Diaporthe* ascogenous stage was produced in culture after several weeks. Species identity of the perfect stage was not determined and this will need further study.

BLACK ROT

Mycosphaerella melonis (Pass.) Chiu & J. C. Walker

During 1960, traces of black rot occurred in Charleston Gray melon shipments from Florida and Georgia. In 1961, *M. melonis* was found associated with *Diplodia* on stem-end decay lesions on Black Diamond melons from Georgia. Black rot, occurring as a blossom-end decay following punctures and other injuries, also caused a 1% loss in a carlot of Charleston Gray from Arkansas.

SCLEROTIUM (PELLICULARIA) ROT

Pellicularia rolfsii (Curzi) E. West

In 1960, 15% loss from decay due to *P. rolfsii* was observed in a car of Charleston Gray melons from Georgia. In 1961, a trace to 1% of this decay was observed on Black Diamond melons from Georgia.

PHYTOPHTHORA ROT

Phytophthora capsici Leonian

Phytophthora rot was observed on Mexican Peacock melons during both seasons. The incidence of disease was generally not greater that 1%, although losses in some cars averaged 6.8%.

Isolations were made from these melons and the Phytophthora cultures readily produced decay on Peacock and New Hampshire Midget watermelons when portions of agar with mycelium were placed on the rinds, without wounding. Sporangial production was profuse within 6 days on these inoculated melons. The extent of decay was similar to that reported previously (5). An isolate obtained in 1961 readily caused stem cankers and fruit infections when inoculated into Early California Wonder pepper plants. Upon this characteristic, together with measurements of sporangia produced on lima bean agar, the organism was identified as *P. capsici* Leonian (1, 6). However, other species have also been recognized as the cause of Phytophthora rot (5).

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STEM-END ROT

Diplodia natalensis P. Evans

During 1960, Diplodia stem-end rot was noted in six different carlots of Missouri Charleston Gray melons. The incidence of this disease in some cars was 16%. The decay appeared to originate mainly through cut stems, followed by a progressive decay into the rind and flesh. Occasional Diplodia rot was found on blossom ends following stem punctures.

In 1961, traces of incipient stem-end rot, shown as softened and discolored stems, were seen on melons from the southern States. The following percentages of advanced stem-end rot were observed: Texas: Charleston Gray 27.3, 19.3, and 9.2; Black Diamond 5.0.
Georgia: Charleston Gray 6.6, 5.0; Black Diamond 5.8.
Oklahoma: Black Diamond 0.5.

GENERAL DISCUSSION

Records of the occurrence of watermelon diseases on the Chicago market during the past 25 years indicate that the total incidence of Diplodia stem-end rot has decreased markedly. However, the above figures suggest that on the Charleston Gray variety stem-end rot may be increasing.

Mycosphaerella black rot has been present during each of the last 6 years, with occasional shipments having losses of about 25%. Phytophthora and Pellicularia rots are of comparatively recent importance on the market (5,4). In recent years anthracnose has occurred in enphytotic amounts, particularly since Charleston Gray has become the most important variety on the Chicago market. However, occasional carlots and truckloads of this variety have shown severe incidence of this disease.

Among the minor diseases, Fusarium rot and Rhizopus rot were found infrequently, following severe bruising or breakage of melons in the cars. In 1961, a Charleston Gray melon from Texas was found with charcoal rot (Macrophomina phaseoli (Maubl.) Ashby) around a puncture wound. Internal rind spot (causal agent undetermined) (3), of Peacock melons from Mexico, was another disease observed in Chicago. The incidence of these necrotic, irregular, rust-colored rind spots seemed higher in 1960 than in 1961, but no definite data were obtained.

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EFFECTS OF PRETREATMENT OF HETERODERA SCHACHTII CYSTS WITH SUGAR SOLUTIONS ON EMERGENCE OF LARVAE IN SUGAR-BEET ROOT DIFFUSATE[1]

Arnold E. Steele[2]

Summary

When cysts of Heterodera schachtii were treated with 0.1 to 60% sucrose for 96 hours and subsequently placed in sugar-beet root diffusate, emergence of larvae was reduced only 40% by the highest concentration tested. Therefore, control of the beet nematode by addition of sugar to soil does not seem practical.

Feder (3) reported that 100% of the nematodes of four species tested were killed by immersion for 1 hour in 40 or 50% sucrose solution. He did not include any species of the genus Heterodera. This paper reports the results of experiments on the effects of sucrose on the emergence of larvae from cysts of the sugar-beet nematode, Heterodera schachtii Schmidt.

MATERIALS AND METHODS

The test consisted of treating groups of 50 cysts with sugar (sucrose) solutions or tap water for 96 hours at room temperatures (70° to 80° F). The sugar concentrations ranged from 0.1 to 60.0% (Table 1).

Table 1. Average number of Heterodera schachtii larvae emerged from cysts which had been pretreated with various sucrose solutions in four replications.

		Number of larvae emerging			
Treatment no.	% sucrose	in sugar solution	in tap water	in beet root diffusate[a]	Total
1	0.1	259	198	8,538	8,995
2	1.0	227	8	8,510	8,745
3	5.0	27	3	8,765	8,795
4	10.0	35	13	8,548	8,596
5	20.0	218	17	9,363	9,598
6	30.0	0	1	9,005	9,006
7	40.0	0	9	8,358	8,367
8	50.0	0	10	7,238	7,248
9	60.0	0	0	6,103	6,103
10	0	---	1,875	10,210	12,085
11	0	---	6,147		6,147
LSD .05				2,300.7	

[a] Figures given for treatments 1-10 are numbers of larvae which emerged in beet diffusate after cysts were pretreated with the indicated sugar solutions. In treatment 11, cysts were immersed in tap water throughout the test.

Each treatment was replicated four times. Groups of cysts which received treatments 6-9 were placed in tea bags and submerged in vials containing the sugar solutions because of the tendency of the cysts to float. Cysts of all other treatments were placed in Syracuse watch glasses. After the initial treatment the cysts were removed and placed in tap water for 24 hours to allow the sugar solutions to diffuse out. Floating cysts and cysts broken in handling were discarded, and the larvae which emerged were counted. Following the tap water treatment, the cysts were rinsed in tap water and placed in watch glasses containing sugar-beet root diffusate or tap water for 6 weeks under conditions described by Golden (4) for other hatching tests. The numbers of larvae emerged in each of the various treatments were adjusted to the equivalent hatch of 50 cysts (Table 1) and analysed for statistical significance by the analysis of variance method.

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RESULTS AND DISCUSSION

Complete inhibition of emergence in sugar solutions occurred in treatments containing 30% or more of sugar (Table 1). After transfer from sugar solution to tap water, significantly fewer larvae emerged from cysts pretreated with 50 or 60% sugar solutions than from cysts treated with water. However, the highest sugar concentration tested reduced the hatch by only 40%.

Fairbairn (2) cited a number of studies wherein eggs of several nematode species were found to be highly impermeable to solutions of salts or sucrose. Dropkin, et al. (1) demonstrated that normal hatching of nematode eggs occurred after removal from inhibiting solutions of salts or dextrose.

The results of the present test suggest that eggs of H. schachtii are relatively impermeable to solutions containing high concentrations of sucrose. Consequently, control of the beet nematode by addition of sugar to the soil is probably not practical.

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EFFECT OF SIMULATED FREEZE-CRACKING ON INVASION OF DRY-ICE-INJURED STEMS OF STANLEY PRUNE TREES BY NATURALLY DISSEMINATED CYTOSPORA INOCULUM[1]

A. W. Helton[2]

Summary

Stems on eight 3-year-old Stanley prune trees were injured with dry ice blocks and the injured surfaces penetrated with diagonal knife cuts. Stems on 12 additional trees were injured with dry ice and left uncut.

Naturally disseminated Cytospora fungi invaded 25% of the dry-ice-injured areas on which the bark had not been penetrated by knife cuts, but none of the invaded areas developed aggressive infections. All of the dry-ice-injured areas that were cut with a knife were invaded, and 25% developed virulent infections.

Since frozen areas with knife cuts were invaded so much more frequently than uncut stems, and since the knife cuts approximated minor wounds associated with winter injury in commercial orchards, it was concluded by inference that most of the Cytospora canker damage in winter-injured prune orchards develops because of wounds resulting from winter injury rather than low temperature killing of the tissues *per se*.

INTRODUCTION

Mechanical wounds have been recognized as adequate entry courts for the invasion of stone fruit trees by Cytospora fungi since the earliest investigations of the Cytospora canker problem in Idaho (3, 4).

More recent investigations have demonstrated that low temperature injury, even in localized areas on vigorous trees, also provides satisfactory invasion courts (1, 2). This was true even if ruptures in the stem surfaces were not readily discernible. In the absence of visible cracks in the injured tissues, however, Cytospora invasion resulted in fewer cases of aggressive disease.

Observations made in commercial plum and prune orchards in Idaho since 1951 have indicated that when winter freezing injury has developed in severe proportions it has generally been accompanied by cracking of the bark on injured trees. Sometimes such cracks have been observed only on trunks and major scaffold branches, but sometimes close scrutiny has revealed very small cracks elsewhere. The smaller ruptures in the stem surfaces were nearly impossible to detect on dormant trees; but, as was the case for large cracks, the smaller cracks began to open with spring growth and thereafter were more easily detected.

Such observations suggested that much of the die-back in the upper parts of affected orchard trees might be due to invasion of minor freeze cracks by the fungi, with subsequent flagging of terminal parts and advance of canker margins down the stems.

Experiments were undertaken to test whether this explanation was justified.

MATERIALS AND METHODS

Dry ice blocks 2 x 3 x 10 cm were cut with a band saw and attached to branches and trunks of 3-year-old Stanley prune trees (*Prunus domestica*).

At the points of attachment the stems were approximately 5 cm in diameter. The blocks were attached with a string around the center and a rubber budding strip at each end. The outer surface of each block was covered with two layers of cheesecloth and two layers of paper towel prior to binding it to the stem. The blocks were bound to the stems of the test trees in no particular arrangement with respect to location.

The test trees were in a vigorous condition and growing on nearly level land.

Except for unfrozen controls, the experiments included two treatments. In treatment A the stems were injured with the dry ice blocks, then wounded on the injured surfaces with a knife blade to simulate small freeze cracks. This was accomplished by drawing a sharp blade diagonally across the frozen area, perpendicular to the stem surface, with sufficient pressure to make a cut approximately 2 mm deep. The knife was disinfested with alcohol between cuts. The stem surfaces were not disinfested in order to simulate natural cracking conditions. The

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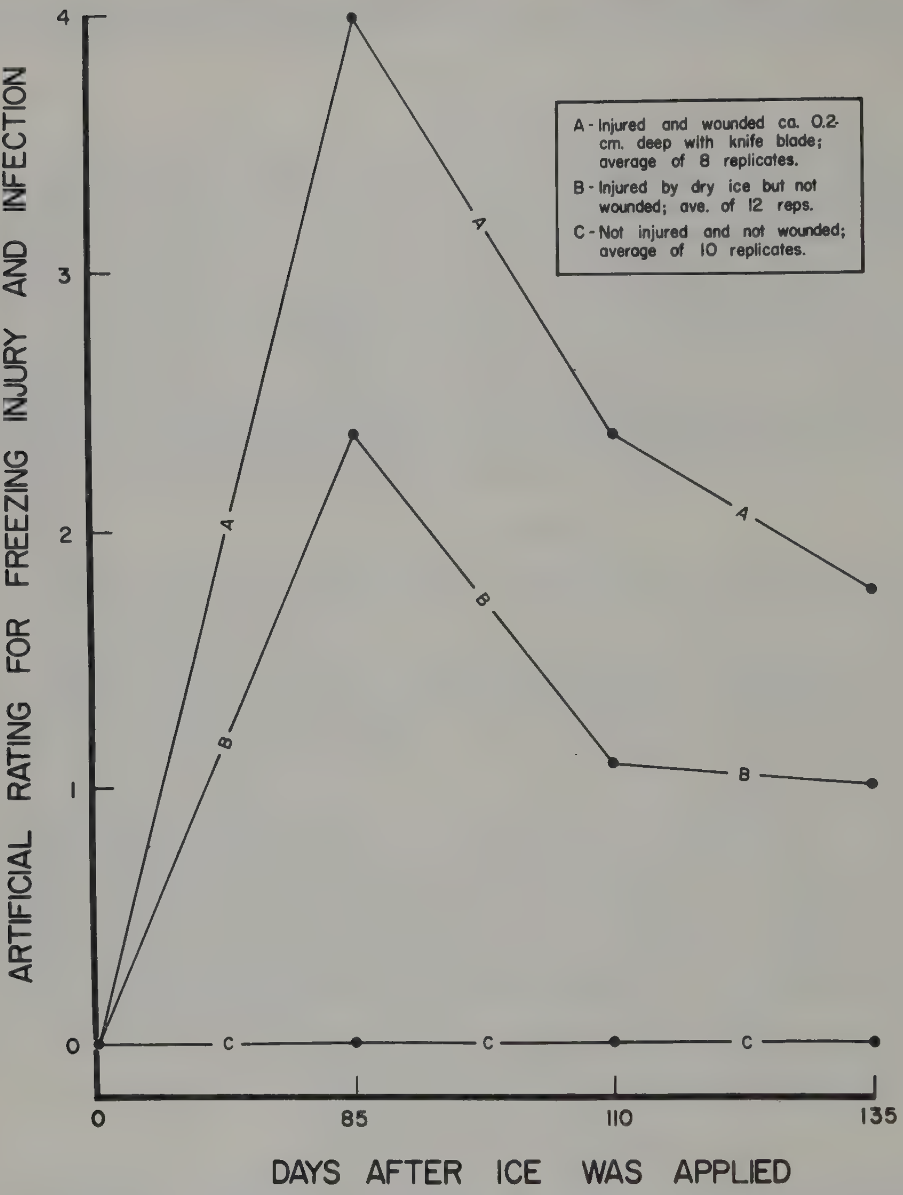

FIGURE 1. Effects of minor mechanical wounding on invasion of dry-ice-injured stems of 3-year-old Stanley prune trees by naturally disseminated Cytospora fungi.

cuts were made 2 hours after evaporation of the dry ice blocks. No side pressure was applied to the blade to open the cuts, which were discernible only as very fine lines. Treatment A was replicated eight times. Treatment B was replicated 12 times and consisted only of freezing of the stem tissues with the dry ice blocks.

Test tree reactions, both to dry ice injury and invading Cytospora fungi, were recorded by means of an arbitrary numerical rating system, as follows: 0) No symtoms, either of dry ice injury or Cytospora invasion. 1) Development of healthy callus around a flat-face caused by the dry ice injury. 2) Flat-face with no appreciable marginal callus. 3) Flat-face lightly pimpled with Cytospora pycnidial pustules. 4) Flat-face moderately pimpled. 5) Flat-face densely pimpled. 6) Gummosis of the flat-face and/or adjacent stem tissues. 7) An expanding Cytospora canker originating from the flat-face. 8) Parts terminal to the infected flat-face flagged, that is, foliage discolored and drooping. 9) All parts terminal to the infected flat-face dead. 10) Canker expanding downward from the infected flat-face. 11) Infected tree dead.

RESULTS

The stem-freezing phase of the experiment was carried out on the morning of March 27, at which time the dry ice blocks evaporated in approximately 1.5 hours.

No symptoms were observed on unfrozen control trees during the course of the study.

Twenty-five percent of the dry-ice-injured areas that were not wounded with the knife blade were invaded by Cytospora strains that behaved in a saprophytic manner (Fig. 1). All of the dry-ice-injured areas that were wounded were invaded, and 25% developed virulent Cytospora canker disease.

DISCUSSION AND CONCLUSIONS

As indicated in Figure 1, the low-temperature-injured stem tissues of the test trees were invaded by Cytospora fungi much more frequently when they were cut with a knife after the injury.

Low temperature winter injury in orchard trees has been observed to affect much more of each tree than one small spot on one branch, as was the case in these experiments. Because of this, it is postulated that both the winter injury, and the subsequent bark stresses due to unequally distributed cambial growth in injured orchard trees, creates a multitude of minute wounds similar to those made by the knife blade as herein reported.

The conclusion was reached, therefore, that most of the Cytospora canker damage that develops in winter-injured orchards develops because of wounds associated with the injury rather than because of cell death per se. Consequently, it is postulated also that varieties more resistant to low temperature injury will be much less affected by naturally disseminated Cytospora fungi even after relatively severe winters.

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EFFECTS OF SEED INOCULATION, SOIL FUMIGATION, AND CROPPING SEQUENCES ON SOYBEAN NODULATION IN SOYBEAN-CYST-NEMATODE-INFESTED SOIL[1]

James M. Epps and Albert Y. Chambers[2]

Summary

Reduced nodulation of soybeans is frequently associated with infection by the soybean cyst nematode, Heterodera glycines, in Tennessee. In experiments near Ridgely during 1957 to 1959, the addition of inoculum of Rhizobium japonicum to planting seed did not significantly increase nodulation in soil heavily infested with the nematode and cropped to soybeans the preceding season. In two of six soil-fumigation experiments in 1958 and 1959, nodulation was significantly increased by fumigation. In 1958, highly significant increases in nodulation were obtained with row applications of granular DBCP at 1, 3/4, and 1/2 gallon/acre at planting and 1/2 and 1/4 gallon/acre sidedressed 3 weeks after planting. In 1959, broadcast applications of D-D mixture at 40 gallons/acre and D-EDB mixture at 12 gallons/acre gave highly significant increases in nodulation while EDB at 4 1/2 gallons/acre gave significant increases. Nodulation did not differ significantly from controls in four other soil-fumigation experiments. Data recorded in 1959 and 1961 from similar 4-year cropping sequence experiments set up in 1956 and 1958 showed highly significant increases in nodulation in a cropping sequence of soybeans, soybeans, cotton, and soybeans as compared with continuous soybeans for 4 years. Nodulation was significantly decreased in a cropping sequence of soybeans, cotton, soybeans, and soybeans in comparison with 4 years of soybeans.

INTRODUCTION

The soybean cyst nematode, Heterodera glycines Ichinohe, 1952, causes stunting and general unthriftiness of soybean plants in Tennessee with a marked yellowing of the foliage which progresses upward from the lower leaves. Symptoms suggest that the plants are deficient in nitrogen, and infected root systems have fewer and smaller nodules than uninfected plants. Severely infected plants may be completely devoid of nodules.

Ichinohe (2) reported a similar reduction in nodulation of soybeans grown in infested soil in Japan. Endo and Sasser (1) stated that nodulation was inversely correlated with "white cyst" numbers in soil-fumigation experiments in North Carolina. Ross (3), also working in North Carolina, found nodulation inhibition in soybeans associated with soybean-cyst-nematode activity. The objective of the present investigation was to study the effects of inoculation of planting seed with nitrogen-fixing bacteria, soil fumigation, and cropping sequences on the amount of nodulation of soybeans grown in soil heavily infested with the soybean cyst nematode.

MATERIALS AND METHODS

Rhizobium Inoculation: Experimental plots were set up in the Mississippi River delta area near Ridgely, Tennessee, in a field known to be heavily infested with the soybean cyst nematode. A commercial formulation of Rhizobium japonicum was added to Lee soybean seed according to the manufacturer's recommendation prior to planting. The seed were planted in single-row plots (3 x 20 feet) on land that had been planted to soybeans the year before the beginning of the experiment. Nodule-index readings were made 4 to 6 weeks after planting. Four plants were rated from each plot on the following scale: 0 = no nodulation; 1 = trace; 2 = light; 3 = moderate; and 4 = heavy. A randomized-block design with four replications of each treatment was used. The experiment was initiated in 1957 and repeated in 1958 and 1959.

Soil Fumigation: 1,3-dichloropropene plus 1,2-dichloroporpane (D-D mixture), 1,2-dibromoethane (EDB), 1,2-dibromo-3-chloropropane (DBCP), and 1,3-dichloropropene plus 1,2-dibromoethane (D-EDB mixture) were applied to soil heavily infested with the soybean cyst nematode 3 to 4 weeks before planting of soybeans. Rates for row treatments were: D-D mixture, 20 gallons/acre; EDB, 2 1/4 gallons/acre; DBCP, 1 gallon/acre; and D-EDB mixture, 6 gallons/acre. Rates for broadcast treatments were: D-D mixture, 40 gallons/acre; EDB, 4 1/2 gallons/acre; DBCP, 2 gallons/acre; and D-EDB mixture, 12 gallons/acre. Row treatments were made at 12-inch intervals in the center of the row, and broadcast treatments were made on a 12-inch grid. Fumigants were applied with a commercial applicator to a depth of 6 inches. Each treatment was replicated four times in a randomized-block design with plots 9 x 20 feet in 1958 and 12 x 30 feet in 1959.

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[2] Nematologist, Crops Protection Research Branch, Crops Research Division, United States Department of Agriculture, and Assistant Professor, Department of Plant Pathology, Tennessee Agricultural Experiment Station, respectively.

In 1958 and 1959, DBCP granules (10%) were applied to the center of the rows to a depth of 3 to 4 inches. The fumigants were covered, and soybeans were planted directly over the treated area. In 1958, DBCP was applied at three rates at planting. The rates were 1/2, 3/4, and 1 gallon of active DBCP per acre. In 1959, the rates were the same except for omission of the 1- and 3/4-gallon rates and the addition of a 1/4-gallon rate. Sidedress treatments were made in furrows 3 to 4 inches deep and 3 to 4 inches to the side of the rows. DBCP granules were applied at 1/4 and 1/2 gallon/acre in both 1958 and 1959. The granules were covered with a push plow. Plot size was 9 x 20 feet in 1958 and 9 x 30 feet in 1959. A randomized-block design with four replications of each treatment was used. The scale and procedure given earlier were used to make the nodule-index readings 6 to 8 weeks after planting.

Cropping Sequences: The amount of nodulation of soybeans following two cropping sequences and continuous soybeans were compared in separate experiments. Four-year sequences were set up in a field heavily infested with soybean cyst nematodes. The first cropping sequence consisted of soybeans in 1956, soybeans in 1957, cotton in 1958, and soybeans in 1959. The second sequence was soybeans in 1956, cotton in 1957, soybeans in 1958, and soybeans in 1959. The methods and scale listed earlier were used to make nodule-index readings in 1959 during the fourth year of each sequence. The cropping sequences were repeated in another location in the same field beginning in 1958, and data were recorded in 1961. A randomized-block design with four replications (plots 12 x 20 feet) of each cropping sequence was used. Nodule-index ratings were made 4 to 6 weeks after planting.

RESULTS

Rhizobium Inoculation: The addition of inoculum of *Rhizobium japonicum* to planting seed did not increase nodulation significantly (Table 1).

Soil Fumigation: None of the preplant row treatments significantly increased nodulation in 1958 or 1959 (Table 2). No significant increases in amount of nodulation were obtained when fumigants were applied broadcast in 1958, but in 1959 D-D mixture and D-EDB mixture gave highly significant increases while EDB gave significant increases (Table 2). All rates of granular DBCP at planting and when sidedressed 3 weeks after planting gave highly significant increases in nodulation in 1958, but none of the treatments were significantly different in 1959 (Table 3).

Cropping Sequences: The difference in nodulation between the soybeans-soybeans-cotton-soybeans cropping sequence and continuous soybeans was highly significant in both years (Table 4). Significantly less nodulation was found in the soybeans-cotton-soybeans-soybeans cropping sequence in the second year following cotton in 1959 and 1961 than in continuous soybeans (Table 4).

Table 1. Mean nodule indexes of soybeans inoculated with *Rhizobium japonicum* and grown in soybean-cyst-nematode-infested soil.

Treatment	Mean nodule index [a] 1957	1958	1959
Inoculation[b]	2.0	1.8	2.8
No inoculation	1.8	1.8	2.5
LSD 5%	N.S.	N.S.	N.S.

[a] Nodule index based on average of four plants from each of four replications using the following scale: 0 = no nodulation; 1 = trace; 2 = light; 3 = moderate; 4 = heavy.

[b] Inoculum was applied to seed before planting at rates recommended by manufacturer.

Table 2. Mean nodule indexes of soybeans grown in soybean-cyst-nematode-infested soil treated with row and broadcast application of various nematocides.

Kind of application and fumigant	Rate (gallons/acre)	Mean nodule index[a] 1958	1959
Row application:			
D-D mixture	20	2.5	1.5
EDB	2 1/4	2.5	2.8
DBCP	1	2.0	2.8
D-EDB mixture	6	3.0	1.8
None	---	1.5	1.2
LSD 5%		N.S.	N.S.
Broadcast application:			
D-D mixture	40	3.0	3.0**
EDB	4 1/2	2.5	2.0*
DBCP	2	3.5	1.2
D-EDB mixture	12	2.5	3.8**
None	---	2.5	0.5
LSD 5%		N.S.	1.1
LSD 1%		N.S.	1.6

[a] See footnote a, Table 1.

Table 3. Mean nodule indexes of soybeans grown in soybean-cyst-nematode-infested soil treated with various rates of granular DBCP at planting and 3 weeks after planting.

Treatment	Rate (gallons/acre)	Mean nodule index[a] 1958	1959
DBCP:			
At planting	1	3.5**	---
	3/4	3.4**	---
	1/2	3.0**	3.0
	1/4	---	2.0
Sidedressed	1/2	3.0**	2.0
	1/4	3.0**	2.0
None	---	2.0	1.8
LSD 5%		0.7	N.S.
LSD 1%		1.0	N.S.

[a] See footnote a, Table 1.

Table 4. Mean nodule indexes of soybeans following two cropping sequences and continuous soybeans in soybean-cyst-nematode-infested soil.

Cropping sequence	Mean nodule index[a] 1959	1961
First:		
Soybeans-soybeans-cotton-soybeans	4.0**	4.0**
Continuous soybeans	2.0	2.5
Second:		
Soybeans-cotton-soybeans-soybeans	0.8	2.9
Continuous soybeans	2.2*	3.6*

[a] See footnote a, Table 1.

DISCUSSION

Inoculation of planting seed with Rhizobium japonicum did not significantly increase nodulation of soybeans grown in soil heavily infested with the soybean cyst nematode and cropped to soybeans the previous season. These results indicated that light nodulation was not due to lack of inoculum in the soil.

In only two of the six soil-fumigation experiments were there significant increases in nodulation, and in these there was a significant decrease in white female numbers noted on the roots of the soybeans. These findings are in agreement with and support the results obtained by Endo and Sasser (1).

Cropping-sequence experiments indicated that planting nematode-infested land to a nonsusceptible crop (cotton) for 1 year gave highly significant increases in nodulation of the soybean crop following cotton. In the second year of soybeans following cotton, however, nodulation of soybeans was significantly reduced. Probably the most credible explanation for this reduction is that nematode numbers were increased during the crop of soybeans following cotton to a level about five times the level in continuous soybeans. The soybean plants following cotton were large and had extensive root systems which were optimum for rapid reproduction of the nematode. In the second year of soybeans following cotton, nematode numbers were very high at planting in comparison with numbers from continuously planted soybeans. There was no consistent relation between white female numbers and the amount of nodulation as in the fumigation experiments.

While the present and previous work have shown that there is often an inverse correlation between infection with the soybean cyst nematode and nodulation of soybeans, the cause of nodulation reduction in soybeans grown in the soybean-cyst-nematode-infested soil has not been established. Endo and Sasser (1) theorized that the mass invasion of soybean roots by nematode larvae interferes with the establishment of the nitrogen-fixing bacteria. The reduction in nodulation is possibly due to a complex of nematodes and root-rot organisms which cause rotting of the soybean roots and a loss of nodules already developing and sites for additional nodules.

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PRELIMINARY REPORT ON A MELOIDOGYNE SP. PARASITE OF GRASS IN FLORIDA

E. B. Sledge[1]

INTRODUCTION

In September 1959 a severe infestation of root-knot nematodes (Meloidogyne sp.) was found on St. Augustine grass (Stenotaphrum secundatum) by the author. Brown patches were noticed on the affected lawn and examination of grass roots from the perimeters of these areas revealed numerous root-knot nematodes. Male, female and larval specimens from the population were sent to Dr. L. G. van Weerdt[2] for examination.

The occurrence of this root-knot nematode on St. Augustine grass was subsequently reported by van Weerdt, et al. (3), who referred to the organism as the grass root-knot nematode and disclosed certain interesting characteristics of the worms. Some of these are:

1. Mature females protrude conspicuously from roots with only head and neck embedded in host tissue.
2. Eggs deposited partly inside the female body and partly in a gelatinous matrix attached to posterior part of nematode.
3. Vulva and anus situated posteriorly on a slight protrusion of the body.
4. Female perineal pattern resembling that of Meloidogyne javanica (Treub, 1885) Chitwood, 1949. Unlike M. javanica, lateral lines not completely interrupting transverse striations in perineal region.
5. Mature female body oval with neck and head situated well to one side of median plane through vulva.
6. Larval tail not sharply pointed as in M. javanica, but conoid as in M. incognita.
7. Larval rectum dilated as illustrated by Chitwood (1) for M. incognita.
8. Infected St. Augustine grass roots without galls, with only a slight swelling at the point of attack.

Studies on the biology and taxonomic status of this nematode are being conducted by the author. The following is a preliminary report of studies on host-parasite relationships.

MATERIAL AND METHODS

Seventeen plant varieties were tested as possible hosts of the grass root-knot nematode. Six plants of each variety were inoculated with chopped Meloidogyne sp. infected St. Augustine grass roots and grown in fumigated soil in 6-inch pots. Six noninoculated plants of each variety served as controls. Plants were maintained in the greenhouse for 30 days. Visual comparisons were made of foliar conditions of the inoculated and control plants during this time. At the termination of the test, root systems of inoculated plants were macerated in Jeffrey's solution after the method of Dropkin, Smith and Myers (2), to reveal nematodes within the tissues. The presence of eggs, larvae, and adult worms was considered proof of completion of the life cycle on a plant variety. Host plants are herein considered to be those on which the nematode's life cycle was completed. The nematode's failure to complete its life cycle on a particular plant variety tested once is not considered conclusive evidence of the plant's being a non-host.

RESULTS AND DISCUSSION

The grass root-knot nematode completed its life cycle on 6 of the 17 plant varieties inoculated (Table 1).

All of these six varieties are members of the family Gramineae. This nematode did not complete its life cycle on three of the grasses tested: Ormond Bermuda, Emerald Zoysia, and Floratine St. Augustine. These varieties are currently being retested. Zea mays var. Golden Cross Bantam was tested twice but there were no indications that the nematode completed its life cycle on this variety. Tomato var. Rutgers was tested three times with negative results. It is interesting that the grass root-knot nematode did not complete its life cycle on any of the seven plant varieties outside the family Gramineae. Further host testing may reveal whether the grass root-knot nematode continues to have a preference for grasses, as indicated by the results of these first tests.

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Table 1. Response of Meloidogyne sp. to various plants.

Plant name	Meloidogyne sp. response[a]
Crabgrass (Digitaria sanguinalis)	+
Tiflawn (T-57) Bermuda (strain of Cynodon dactylon)	+
Ormond Bermuda (strain of Cynodon dactylon)	-
Tiffine (T-127) Bermuda (Tiflawn & 57 Bermuda x Cynodon transvaalensis)	+
Tifgreen (T-328) Bermuda (Cynodon dactylon x C. transvaalensis)	+
Meyer (Z-52 Zoysia (strain of Zoysia japonica)	+
Emerald Zoysia (Zoysia japonica x Z. tenuifolia)	-
Floratine St. Augustine (strain of Stenotaphrum secundatum)	-
Pensacola Bahia (Paspalum notatum)	+
Golden Cross Bantam Corn (Zea mays)[b]	-
Rutgers Tomato (Lycopersicon esculentum)[c]	-
Early Crook Neck Squash (Cucurbita pepo)	-
Scarlet Globe Radish (Raphanus sativus)	-
Denver Half Long Carrot (Daucus carota)	-
California Wonder Bell Pepper (Capsicum frutescens)	-
Duncan Grapefruit (Citrus paradisi)	-
Okinawa Peach (Prunus persica)	-

[a] + = Nematode life cycle completed on the plant. - = Nematode life cycle not completed on the plant.

[b] Tested twice.

[c] Tested three times.

Inoculated roots of all hosts except crabgrass had slight swellings at the site of nematode infection. This is consistent with the observations of van Weerdt, et al. (3) on St. Augustine grass. Diminutive galls (1.5 x 1.5 mm) were found on small (0.5 mm diameter) crabgrass roots. Larger crabgrass roots were only slightly swollen where nematode infection occurred.

There were no noticeable differences in the foliage of inoculated and control plants during the 30 days of the host-testing studies. However, in another test of 4 months' duration using Pensacola Bahia grass, 8 of 10 nematode inoculated plants died, and the remaining 2 were chlorotic and stunted compared with the controls. The root systems of these two surviving inoculated plants were conspicuous for their swollen primary roots and paucity of laterals. Considerable root necrosis was noted. The nematode infection in Bahia grass roots was often as heavy as that observed in St. Augustine grass roots (Fig. 1). Mature female nematodes were so numerous in some cases that their bodies were juxtaposed in the cortex all around the stele in root sections of more than 1 cm in length. When such roots were dissected in water, numerous eggs, infective stage larvae, and male worms issued forth from the root as though under pressure. Developing larvae and adult females usually were found with heads embedded in the stele. Preliminary examination of histological preparations of infected St. Augustine roots has revealed the presence of giant cells.

These findings concerning this nematode's parasitic habit on St. Augustine grass are consistent with those of van Weerdt, et al. (3), except for the mature female's position on the root. On the basis of the material at their disposal, these authors reported that mature females protruded conspicuously from the roots with only the head and neck embedded in the tissue. Examination of large numbers of infected roots indicates that this condition, although occasionally encountered, is exceptional. The head and neck of most females is embedded in the stele and the remainder of the body covered by the cortex.

Attempts were made to control the grass root-knot nematode in a St. Augustine grass lawn. Three applications of 1,2-dibromo-3-chloropropane (commercial DBCP), applied 40 days apart, at the rate of 4 gallons of technical material per acre, followed by 1 acre-inch of water, failed to control this plant parasite. Examination of infected roots at various intervals after each of the treatments revealed no discernible reduction in the population density of the parasites. Approximately 6 months after the last application of DBCP, almost all the St. Augustine grass in the lawn had died. The volunteer native Bermuda grass (Cynodon dactylon) replacing the St. Augustine also became heavily infected with the grass root-knot nematode. After several months, this grass had not grown sufficiently to cover the ground.

The grass root-knot nematode is an extremely interesting parasite, and its possible role as a plant pathogen may be established upon completion of further studies. This Meloidogyne sp. may well become an important economic pest of lawn and pasture grasses.

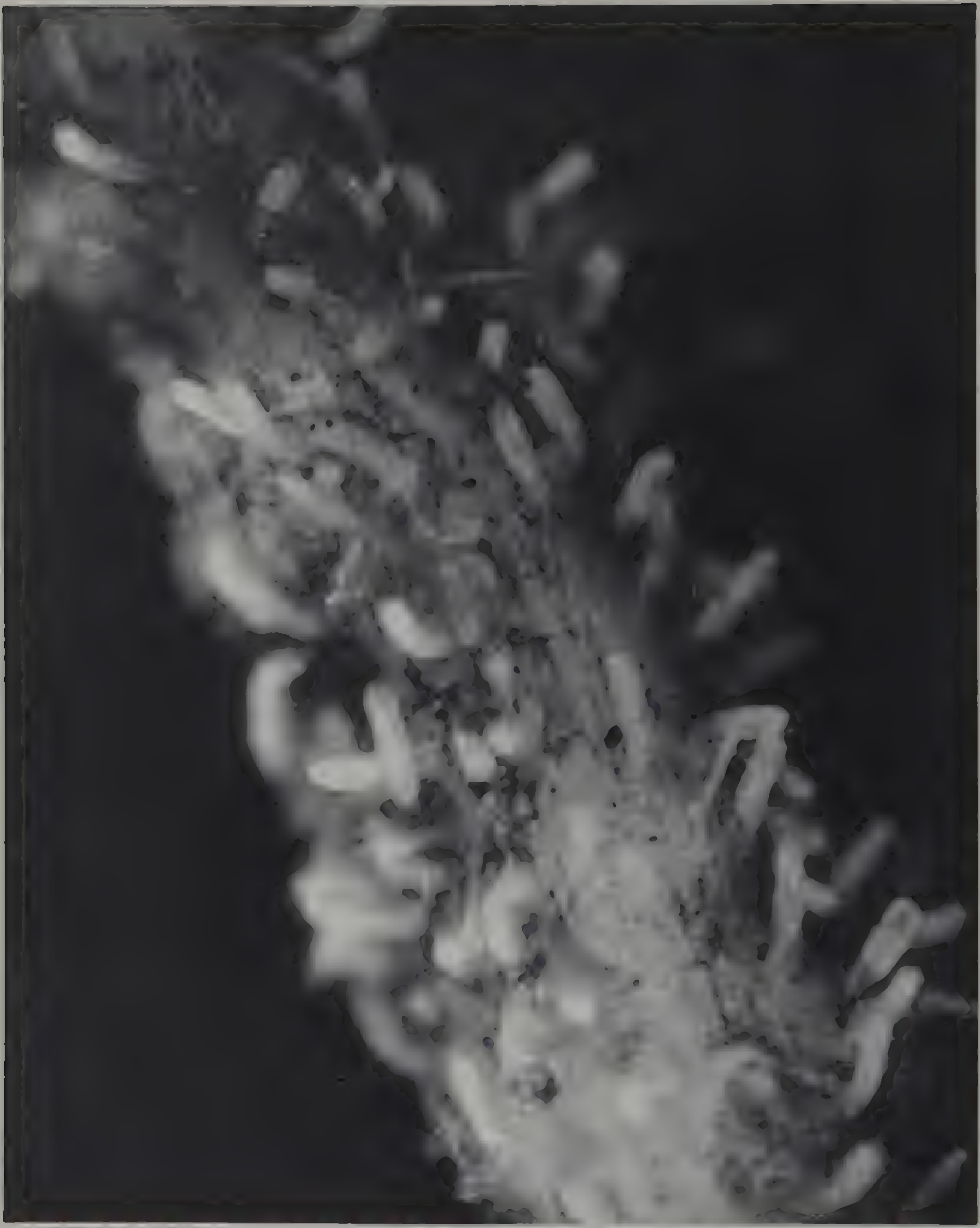

Figure 1. Grass root-knot nematode larvae attached to stele of St. Augustine grass roots.

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SCLEROTINIA BLIGHT OF BAHIA AND COASTAL BERMUDA GRASSES[1]

Douglas C. Bain[2]

Abstract

Sclerotinia homeocarpa F. T. Bennett, the fungus which causes dollar spot of turf grasses, was determined to be also the cause of a disease of Bahia (Paspalum notatum) and Coastal Bermuda (Cynodon dactylon) grasses in pastures in Mississippi. Because of general appearance and occurrence of the disease, the term Sclerotinia blight is suggested. The variety Argentine Bahia was considered to be less susceptible than others that were tested.

The use of Bahia (Paspalum notatum) and Coastal Bermuda (Cynodon dactylon) as pasture grasses has grown to considerable proportions in Mississippi. Bahia grass is also grown for seed production. Both of these crops apparently have been relatively free from disease in Mississippi, although the dollar spot fungus (Sclerotinia homeocarpa F. T. Bennett) has been reported as causing considerable concern for these and other turf grasses in Florida (2, 3) and other southeastern States (5).

In the latter part of August 1956, attention was called to a rather severe disease of Pensacola Bahia grass in south-central Mississippi. Two fields of about 20 acres each were involved; both had been used for grazing earlier in the season but one was later reserved for seed production. The most outstanding appearances of both fields were large areas of dead grass and dead and lodging flowering culms throughout large portions of the fields. Areas in the field being grazed had a somewhat broken linear pattern such as would be obtained by meanderings of grazing cattle; however, this pattern was not so apparent in the field reserved for seed production. It was evident that the grower would harvest little, if any, seed because of premature dying or lodging of culms. Since 1956, numerous other diseased Bahia grass fields have been found in the southern and south-central parts of the State.

Close examination indicated there was killing of foliage and flowering culms only -- the "rhizomes" apparently were not injured. Leaves were fired back from the tips almost (this varied) to the base, but where parts of blades were still green there were occasional irregular to ovate straw-colored or bleached spots with a reddish border. Rather large, irregular, faintly zonate areas or spots occurred along diseased culms. A disease of similar nature and description was also found in August 1956, in Agronomy test plots of Coastal Bermuda in central Mississippi and was so severe in other plots this year that yield data could not be obtained. The disease of both grasses appears to be definitely a "hot weather" type and becomes noticeably severe in August and September. This is somewhat in agreement with observations on the occurrence of dollar spot of turfgrasses in Florida (2).

Several fungi, including Helminthosporium, Rhizoctonia, Colletotrichum, Curvularia, and an apparently sterile fungus, were repeatedly isolated from diseased material -- sometimes more of one than the other but the sterile fungus was always predominant. Numerous inoculation tests by various methods failed to reproduce the disease on either Bahia grass or Coastal Bermuda as it occurred in the field despite occasional infections by Helminthosporium and Rhizoctonia. Eventually, typical symptoms were obtained from infection by the sterile fungus either by placing oat inoculum around the base of young plants or dusting powdered oat inoculum on leaves and culms. Establishment of the sterile fungus as the cause of the disease was fulfilled by Koch's rules of proof.

Studies of the fungus indicated it might possibly be S. homeocarpa F. T. Bennett, the cause of dollar spot of turf grasses (1). Cultures of this fungus were obtained for comparative purposes from Dr. F. L. Howard (creeping bent grass, Agrostis palustris, in Rhode Island), Dr. Homer Wells (Bermuda grass, C. dactylon, in Florida), and Dr. Robert Endo (Bermuda grass in California). Results of cross-inoculation tests with these isolates and that from Bahia grass indicated that all were equally pathogenic to Bahia and Bermuda grasses and that disease symptoms were similar. Cultural characters of the eastern and western isolates were similar but different from the other two. The southern isolates grew faster at all temperatures tried than the eastern and western but the southern isolate from Bermuda grew at a faster rate than the Bahia isolate. None of the isolates grew in 48 hours at 4° C, but by 72 hours the Bahia manifested some growth. Optimal temperatures (in the range of 4°, 16°, 20°, 24°, 28°, and 32° C) for growth of all isolates was 28°; the mean diameters of the eastern and western isolates after 48 hours was 6.3 and 6.8 cm respectively, the southern Bermuda isolate was 8.7 cm, and the Bahia isolate 7.9 cm. According to Bennett (1), the optimum temperature for growth of British and American "strains" he studied was 20° to 25° and "about 30° C," respectively. The similarity of cultural characters and disease symptoms of all isolates indicated that the Bahia grass fungus was S. homeocarpa. Appearance and occurrence of the disease on Bahia and Coastal Bermuda grasses, which differ from those originally described for dollar spot (4), strongly indicated a "blight" and this term is suggested as a name for the disease of pasture grasses.

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[1] Journal Article No. 970, Mississippi Agricultural Experiment Station.

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Results of inoculation tests with seedlings of Pensacola, Argentine, Common, and Tifhi-1 strains or varieties of Bahia grass indicated that all were susceptible but that Argentine was less so than the others.

Although dollar spot has been "completely controlled" on Tiflawn Bermuda (3) by the application of ammonium nitrate (1# or more N per 1000 square feet), other possible control measures for the blight in pastures are being investigated because of its potential seriousness on pasture grasses.

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INOCULATIONS WITH THE SOOTY-BARK CANKER FUNGUS ON ASPEN

aur 35,560

Thomas E. Hinds[1]

Cenangium singulare (Rehm.) Davidson & Cash is consistently associated with sooty-bark canker of aspen (*Populus tremuloides*) in the central Rocky Mountains. Davidson and Cash (2) described the canker, the fruiting bodies on old bark, the classification of the fungus, and its occurrence on aspen in Colorado. Andrews and Eslyn (1) recently reported that this canker is damaging to aspen stands in New Mexico. The purpose of this paper is to record the results of inoculation tests which confirm that *C. singulare* is the causal agent of sooty-bark canker of aspen.

In September 1955, 35 inoculations (including 4 checks) were made on 9 trees in the Roosevelt National Forest, Colorado. In September 1956, 51 inoculations (including 10 checks) were made on 45 trees in the White River National Forest, Colorado. Inoculum consisted of two different isolates of *Cenangium singulare*. Isolate 680 was cultured from inner bark tissue near the margin of a sooty-bark canker on a living aspen. Isolate 681 was cultured from ascospores obtained from apothecia on old cankered bark on a dead tree. Both trees were found on the White River National Forest. The inoculum was grown for 3 months at 20° C on sterile rye. Small pieces of rye containing mycelium of the fungus were placed in the following wounds: 1-inch wide horizontal slit into the inner bark, 1-inch wide slit through the bark into the wood, outer bark broken by bruising, and one to several small puncture wounds into the cambium. Inoculum was placed on one broken branch stub and on two callused-over wounds. The checks were like the inoculations except sterile rye was used. The inoculum and wounds were covered with sterile cotton and secured to the tree by adhesive tape.

The trees were examined 1 year after inoculation and again in the summer of 1960. A summary of the results is shown in Table 1.

Sixty-three percent of the inoculations produced cankers by the end of 1 year. By 1960, 38% of the cankers were apparently healing, 18% were still active, and 44% were on trees that had been girdled and killed by the fungus.

One tree was girdled by isolate 680, whereas five trees inoculated with isolate 681 were killed. Only 47% of the inoculations with isolate 680 produced cankers; 74% were callusing over in 4 to 5 years (Fig. 1, A). Isolate 681 produced cankers from 81% of the inoculations in which

Table 1. Summary of inoculations with *Cenangium singulare* on aspen.

Type of inoculation	Basis (number)	Number of cankers formed after 1 year	Status of cankers in 1960
Roosevelt National Forest (1955)			
Bark slit inoculation	16	16	4 apparently inactive; 12 on 3 dead trees
Bark slit check	2	0	
Wood slit inoculation	15	15	8 apparently inactive; 7 on 2 dead trees
Wood slit check	2	0	
White River National Forest (1956)		7	2 apparently inactive; 5 active
Bark slit inoculation	13		
Wood slit inoculation	13	7	3 apparently inactive; 3 active; 1 on dead tree
Branch stub inoculation	1	0	
Bruise inoculation	6	0	
Puncture inoculation	6	0	
Old wound inoculation	2	0	
Wound checks	10	0	

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it was used; 12% were callusing over in 4 to 5 years. Apparently, the two isolates were not equally virulent, and the inoculated trees differed in their susceptibility to the disease.

One-year-old cankers averaged 15 inches in height and 7 inches in width, with a maximum of 40 inches in height (Fig. 1, B) and 14 inches in width. After 4 years the active cankers averaged 6 feet in height and 18 inches in width, with a maximum height of 12 feet and 29 inches in width (Fig. 1, C). All checks were callused over (Fig. 1, D) and showed no signs of infection.

Infection of aspen by the fungus apparently takes place through recent wounds to either bark or wood. Although the basis is small, results suggest that little infection takes place through branch stubs or old wounds. Reisolations of Cenangium singulare from cankers resulting from the inoculations and the development of apothecia of the fungus on dead bark covering the cankers confirm that this fungus is the causal agent of sooty-bark canker of aspen.

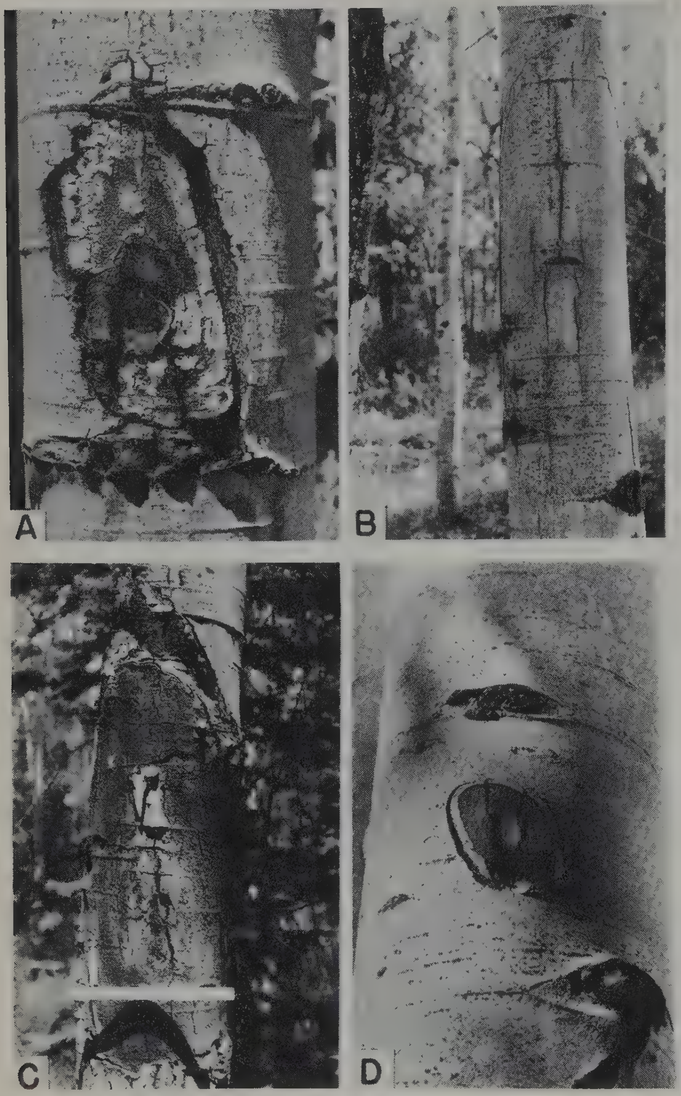

FIGURE 1. Sooty-bark inoculations. A -- Inactive 4-year-old canker callusing over. B -- 40-inch canker formed in 1 year. C -- 4-year-old canker 29 inches in circumference. D -- Callused over check wound.

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A DECLINE CONDITION IN PEARS INDUCED BY PEAR PSYLLA[1]

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Abstract

The insect, pear psylla, has been found to introduce a systemic toxin into pear trees which induces the typical syndrome of pear decline.

The disorder known as pear decline has been of considerable economic importance in the Pacific Coast pear-growing areas in recent years and has been described by various workers (1, 2, 3). Pear decline is associated with sieve-tube necrosis[2] (1) and is much more prevalent where *Pyrus communis* is grafted on oriental rootstocks such as *P. pyrifolia* or *P. ussuriensis*. Heretofore, the causal factors for pear decline have not been elucidated.

The occurrence of pear decline has followed in a general way the spread of the insect pest pear psylla (*Psylla pyricola* Foerst.) on the Pacific Coast. This insect first became a general pear pest in Washington State in the late 1940's. It was adequately controlled by parathion sprays until about 1955, after which time control became difficult and large populations of pear psyllas developed. This increase in pear psyllid population coincided with the large reduction in pear tonnage in Washington which has been attributed to pear decline (1).

Experiments were initiated in 1961 to determine if pear psylla had a causal relation to pear decline. In initial tests pear psyllas were fed on pear trees in the greenhouse for 2 months, then the psyllas were killed and the trees held for observation for an additional 3 months. The test trees included seedlings of Bartlett, *P. ussuriensis*, and *P. pyrifolia* and Bartlett grafted on these three stocks. Histological studies of these trees showed psyllid feeding induced phloem necrosis typical of that reported for pear decline[2] (1). The oriental seedlings were much more susceptible to injury than were Bartlett. For example, there was approximately 15 times as much phloem necrosis in oriental leaf petioles as in Bartlett petioles exposed to similar conditions as evidenced by phloroglucinol-HCl staining. On the grafted trees the Bartlett scions and the Bartlett seedling stock showed relatively little phloem damage, whereas the oriental rootstock showed severe damage below the graft union. In addition to the phloem damage there was evidence of injury to xylem tissue formed at the time of psyllid feeding. Subsequent xylem growth was normal, leaving the injured tissue as a ring. The xylem injury was much more prevalent in the oriental pear tissue.

Translocation studies with carbon-14 and phosphorus-32 confirmed the anatomical studies by showing that psyllid feeding disturbed translocation in trees on oriental rootstocks.

In another test pear psyllid nymphs were caged on individual *P. pyrifolia* leaves at 1, 3 and 10 individuals per leaf. After 2 weeks the leaf petioles were examined histologically. There was a direct relation between the number of psyllas per leaf and the amount of phloem necrosis in the petioles.

This work would seem to implicate a systemic toxin introduced by pear psyllas during feeding as a direct cause of pear decline. Further evidence for a toxin was obtained by homogenizing psyllas in 0.01 M neutral phosphate buffer and immersing stems of freshly cut plants in the homogenate. The presence of a toxin was demonstrated by wilting of the test plants. Similar response was obtained from bean, tomato, *Physalis floridana*, and cucumber as well as pear shoots. *Pyrus pyrifolia* shoots were much more susceptible to the toxin than were those of *P. communis*. Bean was used as a standard test plant for further toxicity studies. Histological examination of bean shoots immersed in psyllid homogenates revealed phloem and pith necrosis. The amount of necrosis was correlated with concentrations of psyllid toxin and proved to be a more sensitive measure of toxin than wilting. Radioactive-phosphorus translocation studies on bean and pear shoots exposed to psyllid homogenates confirmed the histological studies by showing a disturbed translocation in the presence of psyllid toxin. The tracer technique was the most sensitive measure of the toxin effect.

Individual psyllas varied considerably in the amount of toxin they contained. Furthermore, the early nymphal instars were much more toxic than the older nymphs, and adults were the least toxic. In dilution studies, it was demonstrated that only 1/25 of a young or first instar nymph per ml of buffer caused measurable damage to bean by histological examination. With first instar nymphs this amounted to only 0.1g of nymph per ml of buffer.

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[1] Scientific Paper No. 2172, Washington Agricultural Experiment Stations, Project No. 1337.

[2] Henry Schneider. Unpublished data.

Pear psyllid toxin is heat stable and dialyzable. Further studies on the nature of the toxin are in progress.

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INEXPENSIVE TEMPERATURE TANKS[1]

George Fassuliotis[2]

For use in experiments in which the soil temperature requires control within narrow limits, inexpensive temperature tanks have been constructed at the U. S. Vegetable Breeding Laboratory, Charleston, South Carolina. These are an adaptation of the apparatus described by Earley and Cartter in 1945 (1) and differ from tanks previously described (2) for this purpose in that the soil containers are not immersed in a water bath.

The Charleston temperature tanks were made from obsolete models of an upright type of soft drink dispensing machine, which has a 1/4-horsepower cooling unit (Fig. 1). The door and dispensing mechanism were removed and discarded, the cooling mechanism was removed, and the box was placed on a wooden frame about 15 inches high to bring it to a comfortable working level. The box was painted inside with aluminum paint. The cooling coils were carefully bent to a new shape and the evaporator fan was placed at the botton of the box, whereas the compressor was remounted to operate with the box in its new position (Fig. 2). Two cleat receptacles for 600-watt heating elements were mounted on the sides of the box, and a line-voltage thermostat[3] was installed.

The machine was then wired as indicated by Figure 3. Receptacles B and C facilitate repairs and cleaning and permit disconnecting the cooling or heating elements. The fan was connected to operate continuously.

The top of the box was closed by a sheet of 3/4-inch plywood with holes cut as shown in Figure 1 to suspend the soil containers in the air bath. A refrigerator door gasket makes a tight joint between the cover and the box. The soil containers shown are 2-quart enameled irrigators used in hospitals. They are sealed to the plywood cover with a plastic usually sold as a calking or weather stripping compound. The spouts at the bottom were plugged with corks, but plastic hoses leading to a hole in the bottom of the tank could be attached for drainage.

Preliminary adjustment of the thermostat was made with a temporary cover over the holes in the plywood. Final adjustment was made after the containers filled with soil were in place, by removing one or two containers to gain access to the thermostat.

Several of these tanks have been constructed and have functioned very well for over a year in a greenhouse with maximum ambient temperatures of 120° F. Soil temperature stabilized within 24 hours after being placed in the containers, and fluctuated less than 1° C thereafter. The irrigators have been found very satisfactory for growing plants and also for holding clay pots up to the 4-inch size.

Cost of materials for the original tank including the second-hand vending machine was less than $100. The most expensive items were the 18 irrigators and the thermostat.

Aside from ready availability and the needed parts, there is no particular advantage in the vending machines. Any insulated box and adequate cooling unit will serve the purpose equally well.

Additional units have been built for use in an air-conditioned space where it was desired to maintain a soil temperature above the air temperature[4]. These use only a simple thermostat and heating cable in the bottom of the box. A fan was found to be unnecessary in boxes 24 x 36 x 12 inches. Instead of the irrigators, 2-quart tin cans painted with asphalt were used. Thermographs placed in the soil in the various cans indicated that the temperature was maintained within 1° C.

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[1] Cooperative investigations of Nematology Investigations and the U. S. Vegetable Breeding Laboratory, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Charleston, South Carolina.

[2] Nematologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Charleston, South Carolina.

[3] Minneapolis-Honeywell type TA42B.

[4] These were designed by Mr. Ronald Myers and Dr. V. H. Dropkin at Beltsville, Maryland.

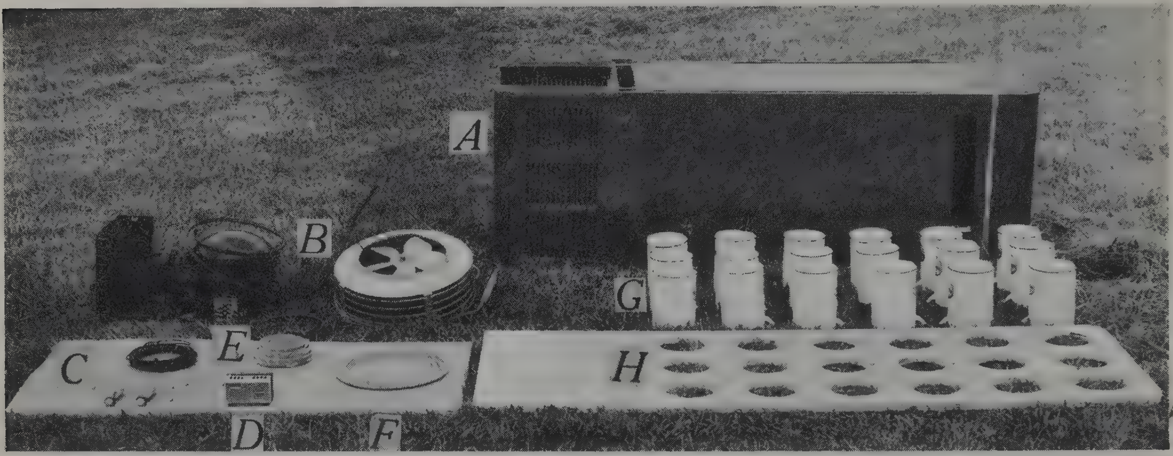

FIGURE 1

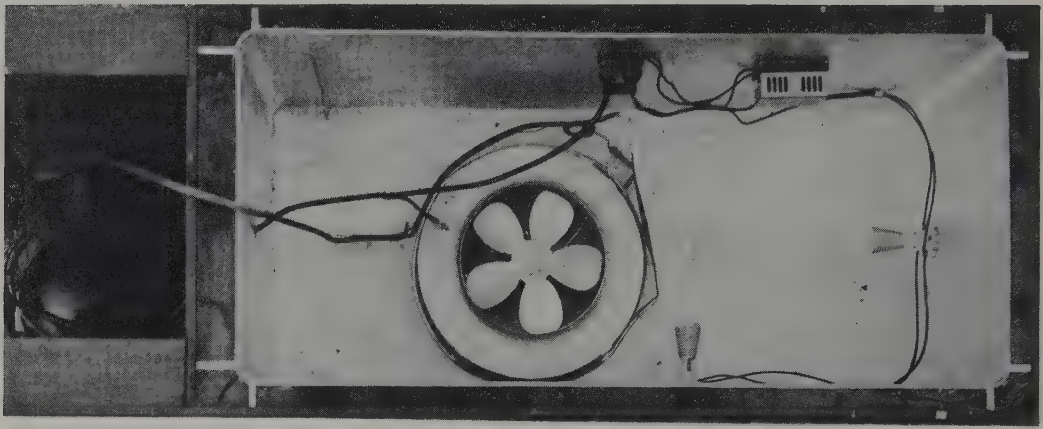

FIGURE 2

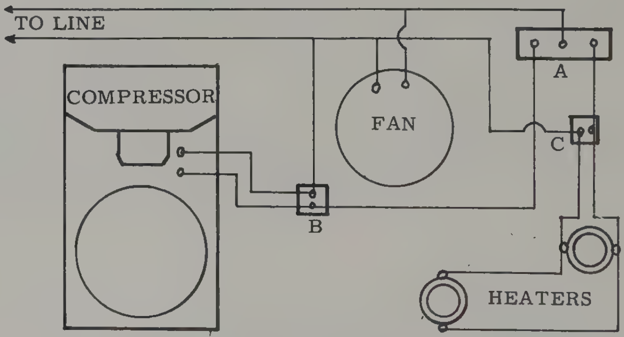

FIGURE 3

FIGURE 1. Components used in assembling temperature tank: A -- Soft drink box. B -- Compressor with attached evaporator fan. C -- Heaters, cleat receptacles, and wire. D -- Thermostat. E -- Calking compound in strips. F -- Refrigerator door gasket. G -- Irrigators. H -- Plywood rack to hold irrigators.

FIGURE 2. Top view of temperature tank showing placement of compressor, evaporator fan, heaters, and thermostat.

FIGURE 3. Wiring diagram. A -- thermostat. B and C -- receptacles and plugs to permit disconnecting heating and cooling units.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, CHARLESTON, SOUTH CAROLINA

A LABORATORY METHOD OF OBTAINING SPOROPHORES OF FOMES ANNOSUS

John S. Boyce, Jr.

A brief survey of the literature[1] has failed to disclose a description of the following method for obtaining sporophores of Fomes annosus (Fr.) Cke. in the laboratory. This fungus is receiving considerable attention because it causes a damaging root and butt rot of conifers in many parts of the world. The method may be useful for growing sporophores not only of F. annosus but also of other wood-decaying Basidiomycetes.

Sporophores of F. annosus were obtained in the following way. A shortleaf pine (Pinus echinata) about 5 inches in diameter at breast height was cut September 27 at Athens, Georgia. The upper bole was sawn into sections 12 inches long with diameters inside bark of 2 to 4 inches.

The freshly cut ends of the bolts were sprayed with a water suspension of F. annosus conidia from a 2-week-old culture on potato-dextrose agar. The isolate had been obtained earlier during the summer from a decayed root of a living windthrown slash pine (P. elliottii). After inoculation, a polyethylene plastic bag was slipped loosely over each end of the bolt and held against the bark with a rubber band (Fig. 1). No effort was made to use aseptic conditions for the inoculations.

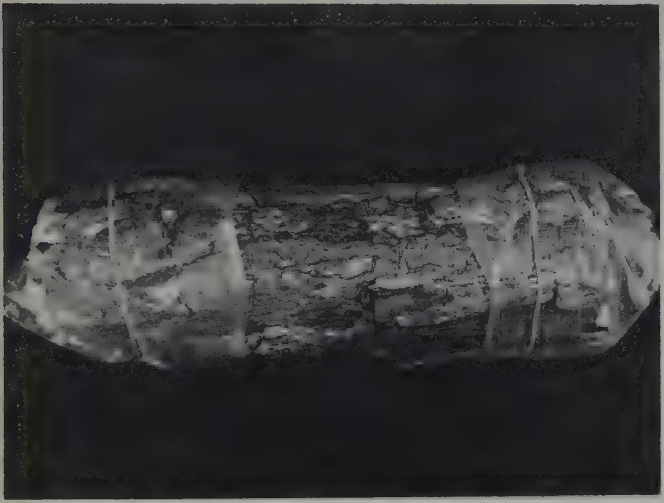

FIGURE 1. Plastic bags attached to pine bolt after inoculation with Fomes annosus.

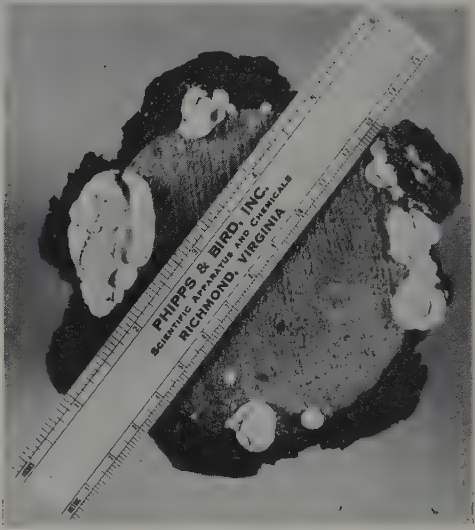

FIGURE 2. Fomes annosus sporophores on pine bolt 7 weeks after inoculation.

The bolts were then incubated at about 70° F in indirect daylight in the laboratory. Abundant F. annosus mycelium bearing typical conidiophores appeared on the inoculated surfaces 1 week later. After 5 to 6 weeks, dense irregular masses of mycelium were present on the bolt ends where the sapwood joined the bark. Some masses had indistinct pores. By 7 to 8 weeks, many mycelial masses had formed mature resupinate sporophores (Fig. 2). Basidiospores were present at this time.

Mature sporophores were produced on 10 of the 14 ends of 7 bolts that were inoculated. The largest separate sporophore 8 weeks after inoculation was 2 x 4 cm although several bolts had more or less continuous sporophores that extended around the sapwood circumference. They formed on bolts 2 inches in diameter as well as on those 4 inches thick. When one bolt was split and wood chips from the inside faces were cultured, it was found that F. annosus mycelium had grown throughout the length of the bolt during a 7-week period.

DEPARTMENT OF PLANT PATHOLOGY AND PLANT BREEDING, COLLEGE EXPERIMENT STATION, UNIVERSITY OF GEORGIA COLLEGE OF AGRICULTURE EXPERIMENT STATIONS, ATHENS

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[1] Koenigs, J. W. 1960. Fomes annosus: A bibliography with subject index. Southern Forest Experiment Station Occasional Paper 181.

FOMES ANNOSUS INFECTION THROUGH PRUNED BRANCHES OF SLASH PINE

Wesley Witcher[1] and Robert E. Beach[2]

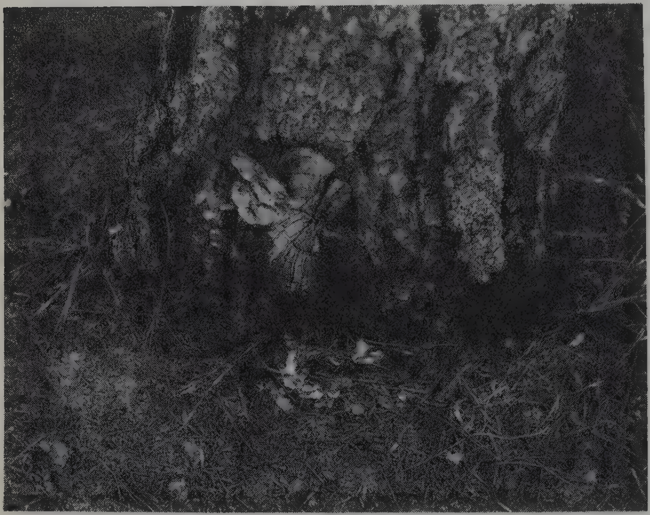

FIGURE 1. *Fomes annosus* fruiting at the base of a pruned slash pine tree.

Conks of *Fomes annosus* Fr. were found in May 1961 on trees in approximately 3 acres of a 17-acre plantation of slash pine (*Pinus elliottii*) belonging to the Graniteville Co. and located 2 miles southeast of Graniteville, South Carolina. The stand was planted during the 1943-1944 planting season and had not been thinned. A large number of trees in the stand had galls caused by *Cronartium fusiforme* Hedgc. & Hunt. Many of the trees in this 3-acre area were pruned in the winter of 1956 to eliminate branches with fusiforme galls. Pruning wounds ranged from 1 to 3 inches in diameter and from 3 inches to 3 feet from the ground. Some of the limbs were pruned with saws and others with axes. Six infection centers of 4 to 10 trees each were confined to the area in which pruning was done. Conks were found at the base of trees in each of the six centers and on some of the trees bordering the centers. Pruning wounds were found on trees with *F. annosus* conks in each of the infection centers (Fig. 1). Another stand of the same age, separated from the pruned stand by a road, had not been pruned and in it there was no evidence of *F. annosus* infection.

The fungus had apparently invaded through the pruning wounds and spread from there by root contact to surrounding trees. Proof of this mode of entrance has not yet been established but is under investigation.

SOUTH CAROLINA AGRICULTURAL EXPERIMENT STATION, CLEMSON AND STATE FORESTRY COMMISSION, AIKEN, SOUTH CAROLINA

---

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COVERED SMUT OF CRABGRASS

Edgar C. Tullis

Tilletia barclayana (Bref.) Sacc. & Syd. (*Tilletia pulcherrima* Ell. & Gall.) was found in infected ovaries of *Digitaria ischaemum* in lawns near Braddock Heights, Maryland in the fall of 1961. This smut was previously reported on *D. sanguinalis* from Mississippi.

In most infected plants only occasional florets were affected in an inflorescence. The smutted kernels were two to three times normal size in many instances and could be identified in the immature stage by their greater size as compared with the normal florets and also by their bright green color. When mature, the sori were dark brown and frequently ruptured, liberating the spores. The chlamydospores were dark brown, 10 to 29μ in diameter with a hyaline envelope covering the spines. The chlamydospores germinated to produce many primary filiform sporidia which did not fuse. The writer wishes to thank Alice Robert for culturing the smut so that spore germination could be observed.

FREDERICK, MARYLAND

PEANUT RUST IN VIRGINIA

Grover C. Smart, Jr.

A rust of leaves, stems, and branches of peanut was observed on two farms, Southampton County and Nansemond County, in Virginia on October 6, 1961. Plants in small scattered areas of initial infection were killed. To avoid severe losses from the rust disease, the growers harvested their plants prematurely. This is the first known report of a rust on peanut in Virginia.

The rust was identified as Puccinia arachidis Speg. by C. R. Benjamin, The National Fungus Collections, Beltsville, Maryland; L. S. Olive, Columbia University, New York, N. Y.; and J. W. Baxter, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin.

VIRGINIA AGRICULTURAL EXPERIMENT STATION,
TIDEWATER RESEARCH STATION, HOLLAND, VIRGINIA

PEANUT RUST IN NORTH CAROLINA

J. C. Wells

Leaf rust (Puccinia arachidis) has been found affecting peanuts in Hertford, Gates, Perquimans, Martin, Edgecombe, Chowan, Northampton, Bertie, Beaufort, Pitt, Halifax and Greene counties in North Carolina. This is the first recorded report of this disease in this State. Areas of infection ranged from a few feet in diameter to 1/2 acre in size. Owing to the dry condition late in the season, there appeared to be very little secondary spread. Localized areas of infection were reported from Florida in 1931 and Texas in 1941. The disease occurs in various sections of South America and quite generally throughout the West Indies. It appears only in the uredospore stage (stage II) and is often destructive in the West Indies. So far as is known, it does not live overwinter in North Carolina since it does not survive on dead peanut vines or in the soil. Also, no other host of this pathogen is known; consequently, the only source of infection is from spores blown in from the sub-tropcial areas where peanuts survive the winter. Since rust spores can be transported great distances by wind currents, it is most likely that the present infection was blown in from the sub-tropical areas where the disease occurs every year and frequently causes severe losses.

When the rust disease appears toward the middle of the season, it does very little damage, but with an early infection, especially on wet soil, considerable defoliation, premature ripening and a large proportion of shriveled kernels may result. The disease is more prevalent in wet seasons.

DEPARTMENT OF PLANT PATHOLOGY,
NORTH CAROLINA STATE COLLEGE, RALEIGH, NORTH CAROLINA

THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 46 Number 2

February 15, 1962

Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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Paul R. Miller

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THE PLANT DISEASE REPORTER

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Plant Industry Station, Beltsville, Maryland
February 15, 1962
Number 2

CONTENTS

1. The role of seed transmission in the epidemiology of bean mosaics in central Washington
R. S. ROBERTSON, Jr. 71

2. Efficacy of five fumigants for the control of Agrobacterium tumefaciens at various depths in the soil
ROBERT S. DICKEY 73

3. Effect of Bordeaux spray and plastic covering in preventing invasion of low-temperature-injured stems of prune trees by Cytospora fungi
A. W. HELTON 77

4. A comparison of apple virus inoculum from stem pitted, dapple apple, flat limb, and mosaic infected trees
R. C. McCRUM and M. T. HILBORN 80

5. Nonpathogenicity of Pratylenchus penetrans to sterile low-bush blueberry seedlings
R. C. McCRUM and M. T. HILBORN 84

6. Root grafting of city-planted American elms
E. B. HIMELICK and DAN NEELY 86

7. On the attempted induction of resistance in oats to crown rust race 264 by artificial irradiation
M. D. SIMONS, et al. 88

8. Root-lesion nematodes in English holly (Ilex aquifolium) in British Columbia
J. E. BOSHER and W. R. ORCHARD 92

9. Reduction of susceptibility to cucumber scab by cucumber mosaic virus
H. J. HOPEN and D. J. deZEEUW 93

10. Bacterial leaf stripe of sweet corn
C. WEHLBURG 98

11. Differential sensitivity to sodium orthophenylphenate by biphenyl-sensitive and biphenyl-resistant strains of Penicillium digitatum
PAUL R. HARDING, Jr. 100

12. Oil-spray trials on Cercospora diseases of tobacco, peanut, zinnia, and gramalote grass
L. CALPOUZOS, et al. 105

13. Tree killing by Fomes annosus in a genetics arboretum
ROBERT V. BEGA 107

14. Glomerella cingulata and Myrothecium spp. in seeds of small grains
W. F. CROSIER 111

15. Leafhopper vectors of virus diseases in Michigan
J. R. HOFFMAN and O. TABOADA 114

16. Relation between the effect of biphenyl on germ tube development and control of citrus green mold among isolates of Penicillium digitatum
RUBEN DURAN 115

17. Culture of apical meristems of Fragaria vesca strawberry plants as a method of excluding latent A virus
R. O. BELKENGREN and P. W. MILLER 119

18. Fungi involved in the seedling disease complex of cotton in the Yazoo-Mississippi Delta
C. D. RANNEY 122

19. Seasonal variation in the incidence of radish yellows and effect of crop rotation on plant survival and yield
J. D. WILSON 124

20. The cause of potato russet scab in Minnesota
M. D. HARRISON and CARL J. EIDE 128

21. Rockingham, a new tomato variety resistant to late blight
A. E. RICH, et al. 130

22. Book Review (Color Handbook of Citrus Diseases)
PAUL R. MILLER 130

23. Book Review (A Monograph of the World Species of Hypoxylon)
JOHN A. STEVENSON 131

THE ROLE OF SEED TRANSMISSION IN THE EPIDEMIOLOGY OF BEAN MOSAICS IN CENTRAL WASHINGTON[1]

R. S. Robertson, Jr. [2]

Abstract

Epidemiology studies of bean mosaic diseases were made from 1955 to 1959. All of the viruses studied were transmitted in some form to some of the seeds of some of the bean varieties tested, and thence to the plants grown from those seeds. Thus, the viruses would be able to survive the winter, between growing seasons. Plants grown from virus-carrying seeds can act as sources of virus inoculum for potential migrant aphid vectors.

Studies of the epidemiology of bean mosaics in field beans were conducted at the Irrigation Experiment Station in south-central Washington's Yakima Valley during the summers of 1955 through 1958. Correlated greenhouse studies were made at the Main Experiment Station, Pullman, Washington between 1955 and 1959. The author (4) concluded that migrant aphids, particularly the green peach aphid, Myzus persicae (Sulzer), were responsible for the spread of the bean mosaics present during the growing season. The author (5) further concluded that some of these viruses were likely persisting between growing seasons in red clover and sweetclover plants. Incidental to this study, it was discovered that the viruses involved were sometimes transmitted to the seed and thence to the next generation of bean plants.

In 1918, Reddick and Stewart (3) reported that bean common mosaic was frequently transmitted through the seed. However, reports of seed transmissibility of other bean mosaics have been rare. Corbett (1) reported a seed-transmissible bean yellow mosaic, and Skotland and Burke (7, 8) discovered that one of the virus entities studied by the author (as "D" virus (4)) was seed transmitted at least in the Columbia Pinto bean variety.

In the findings reported here, some virus-infected beans in the greenhouse were permitted to mature, the seeds thus produced being held for 4 to 12 months before being planted. When such seeds produced virus-infected plants, the mosaic symptoms were much milder than was the case in the parent plant. However, when inoculations with expressed sap, using carborundum as an abrasive (2), were made from the plant infected with seed transmitted virus to healthy test bean plants, the symptoms induced in the test plants were commonly more severe. (Symptoms in infected test plants usually developed 10 to 15 days after inoculation.) The rate of seed production was less in the virulent plants than in healthy check plants. Pods were fewer and usually smaller. The bean virus entities involved here were isolated by Skotland (6) and referred to by the author (4) as bean viruses "A", "B", "C", and "D". The symptoms induced by these viruses have already been considered in earlier papers (4, 5).

Seeds from Virus "A"-Infected Beans: One 7-month-old group of six Red Mexican seeds produced four plants, all apparently virus-free. Lots totalling 195 Early Pink bean seeds, 10 to 11 months old, produced 182 plants, none with mosaic.

However, a 12- to 18-month-old group of 29 Early Pink seeds produced 27 plants, 3 of which had a mild foliage mottling. Carborundum-and-sap inoculations with this mottled foliage produced severe mosaic symptoms in 37 of 50 test Early Pink beans.

A 4-month-old group of 21 Pink seeds all germinated; 4 of the 21 had a mild foliage mottling. Carborundum-and-sap inoculations to 50 test Pink beans produced severe mottling and necrosis in 47 of them. The same results were obtained in 46 of 50 beans when green peach aphids were used as the transmitting agent. Adult aphids, raised on virus-free radishes, were starved for 1 hour, placed on the virulent foliage for 10 minutes, then moved to the test plants (five aphids per plant) for 1 hour.

Seeds from Virus "B"-Infected Beans: One 6-month-old group of 32 Red Mexican seeds all germinated; none of the plants was mottled. A 10-month-old group of 42 Early Pink seeds produced 18 plants, none mottled.

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[1] Most of these data are taken from a thesis submitted to the Graduate School of Washington State University, Pullman, Washington, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Scientific Paper No. 2178, Washington Agricultural Experiment Stations, Pullman, Project 1221. Cooperative Regional Research Project W-12.

[2] Present address: San Jose State College, San Jose, California.

A 12- to 18-month-old group of 60 Early Pink seeds produced 54 plants, 4 mottled. Carborundum-and-sap inoculations from these mottled plants infected 67 of 100 Early Pink beans, the symptoms being a leaf rolling and mild mottling, different from the usual "B" virus symptoms. Perhaps the original "B" virus entity had been altered during the process of seed transmission.

Seeds from Virus "C"-Infected Beans: One 7-month-old group of 14 Red Mexican seeds and an 11-month-old group of 79 Early Pink seeds produced 14 and 62 plants, respectively, none mottled.

One 12- to 18-month-old lot of 35 Early Pink seeds produced 30 plants, 6 mottled. Sap-and-carborundum inoculations with this mottling produced various mosaic symptoms in 42 of 65 Early Pink test plants.

Seeds from Virus "D"-Infected Beans: Skotland and Burke (8) and the author (4) have already indicated the seed transmissibility of this virus in the Columbia Pinto bean variety. The author's greenhouse research supports this conclusion.

One 10-month-old lot of eight Red Mexican seeds produced seven plants, none mottled. A 10-month-old group of five Pinto U. I. #111 seeds produced five plants, none mottled.

One 9-month-old lot of 40 Columbia Pinto seeds produced 31 plants, 14 of which were mottled. Sap-and-carborundum inoculations with this mottled leaf tissue produced typical mosaic "D" symptoms in 48 of 50 Pink beans.

DISCUSSION AND CONCLUSIONS

For every virus entity tested, some seed transmission was found; in some instances the virus may have been altered by its passage through the seeds or by the time element. Positive results were limited to just one bean variety for each virus; however, tests involving other varieties were quite small or lacking altogether, so seed transmission may well be possible in other bean varieties. It can be concluded that all of these bean mosaic viruses, persisting as they can in some bean seed, can thus be transmitted from one generation of bean plants to the next, and thus provide initial (as well as later) bean mosaic virus inoculum to potential migrant aphid vectors.

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WASHINGTON AGRICULTURAL EXPERIMENT STATION, PULLMAN

EFFICACY OF FIVE FUMIGANTS FOR THE CONTROL OF AGROBACTERIUM TUMEFACIENS AT VARIOUS DEPTHS IN THE SOIL

Robert S. Dickey[1]

Summary

Five soil fumigants, 1,3-dichloropropene-1,2-dichloropropane, 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, sodium methyldithiocarbamate, and methylbromide-chloropicrin, were tested for their ability to reduce the population of Agrobacterium tumefaciens at 3, 9, and 15 inches below the soil surface. The reduction in number of A. tumefaciens in artificially infested soil was greater than 99% at all depths for methylbromide-chloropicrin (2 and 3 pounds/100 square feet) and at 9 inches for sodium methyldithiocarbamate. A reduction of approximately 86 and 70% was recorded for sodium methyldithiocarbamate at 3 inches and 1,2-dibromo-3-chloropropane at 9 inches, respectively. None of the fumigants effectively reduced the numbers of the bacterium in buried galls on tomato stems.

INTRODUCTION

An increasing number of nurserymen in New York State are applying soil fumigants for the control of nematodes in nursery soils. The possible control of the crown gall disease by soil fumigation also is a prime consideration for the production of disease-free nursery stock. Therefore, these studies were made to determine the effect of five commonly used soil fumigants on the population of the crown gall bacteria at various depths in the soil.

The effect of soil fumigants on phytopathogenic bacteria has been reported by several investigators (1, 6, 8, 9, 10, 12, 13). Chloropicrin and methyl bromide have been tested for the control of Agrobacterium tumefaciens (7, 11).

MATERIALS AND METHODS

The bacteria were grown in 3000-liter Erlenmyer flasks containing 300 ml potato-dextrose-peptone broth and incubated at 27°C for 48 hours. The cells were harvested by high-speed centrifugation and washed three times in sterile distilled water. The bacterial suspension was atomized onto steam-sterilized loam soil at the rate of 200 ml of suspension per 1500 g of soil. The infested soil was thoroughly mixed and approximately 50 g was placed in 2 1/2 x 2 1/2 inch nylon bags. The bags were stapled closed and a piece of wire was wrapped around the individual bags. The wire extended 3, 9, or 15 inches from the bag and a celluloid label was attached to the free end which served to mark the location of the bag in the field.

The stems of Bonny Best tomato plants were inoculated with A. tumefaciens between the cotyledons and first true leaves. The galls were removed 45 days after inoculation by cutting the stem about 1/4 inch above and below the gall. The excised galls were placed in the middle of nylon bags containing 50 g of steam-sterilized loam soil. The bags were stapled and the wires with labels were attached.

The nylon bags containing infested soil or gall tissue in steamed soil were immediately buried in field plots of silt loam soil which previously had been disced and cultivated. The bags were placed 3, 9, or 15 inches below the soil surface in holes prepared by a post-hole digger. The holes were filled by thoroughly tamping the soil as it was gradually replaced.

The soil was fumigated 1 day after the bags containing gall tissue were buried. Fumigants used were 50% 1,3-dichloropropene-50% 1, 2-dichloropropane (D-D), 41% 1,2-dibromoethane (Dowfume W-40), 67.2% 1,2-dibromo-3-chloropropane (Nemagon EC-2), 32.7% sodium methyldithiocarbamate (VPM), and 98% methylbromide-2% chloropicrin (MC-2). The application rate, injection centers, and treatment following injection are given in Table 1. MC-2 also was applied in a separate test at the rate of 1,2, and 3 pounds per 100 square feet; only bags containing artificially infested soil were used. The liquid fumigants were applied at 6-inch depth with a hand injector. MC-2 was applied under polyethylene tarpaulins. The treatments were replicated four times. The soil temperature was 62° F and the soil moisture content was 22.7 to 23.0% (based on dry soil). The silt loam was composed approximately of 24% clay, 61% silt, and 15% sands, as determined by the hydrometer method (3). The organic matter content was 3%.

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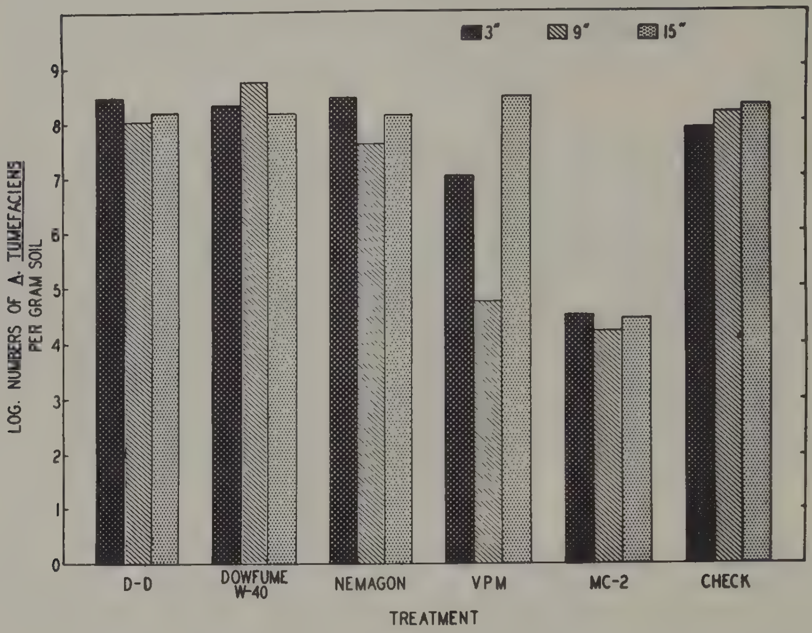

FIGURE 1. Effect of five fumigants on the numbers of *Agrobacterium tumefaciens* in artificially infested soil at 3, 9, and 15 inches below the surface. Application rates of fumigants given in Table 1.

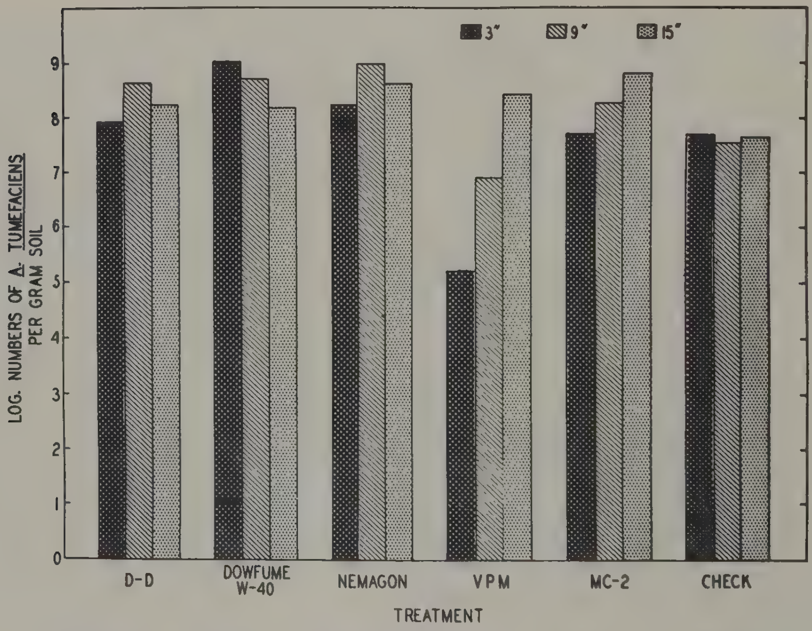

FIGURE 2. Effect of five fumigants on the numbers of *Agrobacterium tumefaciens* in soil surrounding tomato galls at 3, 9, and 15 inches below the surface. Application rates of fumigants given in Table 1.

Table 1. Application rate, injection centers, and treatment following fumigation, for five soil fumigants tested for control of Agrobacterium tumefaciens.

Treatment	Rate	Injection center (inches)	After treatment
D-D	50 gal/acre	10	Light raking
Dowfume W-40	18 gal/acre	10	do.
Nemagon	5 gal/acre	10	do.
VPM	1 1/2 qt/100 square feet	5	18 gal water/100 square feet
MC-2	3 lb/100 square feet	--	Covered 48 hours

The bags of the first replicate were removed 14 days after fumigation and the remaining replicates were tested at various intervals thereafter. All tests had been completed by 142 days after fumigation. The numbers of crown gall bacteria per gram of soil was determined by the standardized plate count technique and pathogenicity test previously described (4). The soil immediately surrounding the gall tissue in the bags was used to determine the numbers of A. tumefaciens. In cases where the gall tissue was not intact, but had broken down, the tissue was separated from the soil as much as possible.

RESULTS AND CONCLUSIONS

The numbers of crown gall bacteria in artificially infested soil following treatment are shown in Figure 1. The reduction in numbers of A. tumefaciens was greater than 99% at all depths for MC-2 and at 9 inches for VPM. A reduction of approximately 86% and 70% was recorded for VPM at 3 inches and Nemagon at 9 inches, respectively. VPM evidently did not penetrate to the 15-inch depth, or the concentration was not sufficient to affect the numbers of the crown gall organism. The results indicate that Nemagon may be effective at an increased rate of application.

MC-2 was as effective at the rate of 2 pounds/100 square feet as at 3 pounds; but it failed to reduce the numbers of A. tumefaciens at the 1-pound rate. These results agree with those reported by Munnecke and Ferguson (11).

The numbers of crown gall bacteria generally were greater in the soil surrounding gall tissue after fumigation than the check (Fig. 2). There was a correlation between the numbers of crown gall bacteria in the surrounding soil and the breakdown of the gall tissue. The galls of the VPM treatment at 3 inches were firm when removed from the soil as compared with the breakdown and sloughing off of tissue for the same treatment at 15 inches, the other treatments, and the check. The deterioration of the gall tissue after 14 days was especially rapid in the soil treated with D-D (9 and 15 inches), Dowfume W-40 (3 and 9 inches), and MC-2 (9 and 15 inches) and is somewhat reflected in Figure 2. These results indicate that the breakdown of the gall tissue was releasing viable bacteria into the soil; but the results do not necessarily imply the multiplication of A. tumefaciens in the soil. However, it appears that the release of A. tumefaciens into fumigated soil was conducive to the survival of the pathogen because the numbers usually were greater than those of the check. This may be attributed to the reduction in antagonism due to the action of the fumigants (5), being similar to the effect demonstrated for steam sterilization (4).

The failure of the fumigants to penetrate adequately the infected tissue probably accounts for the lack of a reduction in the numbers of crown gall bacteria, because some fumigants were effective against the bacteria in artificially infested soil (Fig. 1). McKeen reported the survival of Pseudomonas tomato in lesions of tomato fruit following treatment with methyl bromide and suggested that it possibly was due to the inability of the fumigant to penetrate the host substrate (8).

The results reported here for the effect of methyl bromide (MC-2) on buried gall tissue do not agree with those of Munnecke and Ferguson (11). The fact that flats of soil containing pieces of gall tissue were used by the latter investigators (11) rather than entire galls buried in the field, as well as differences in soil types and method of determining the presence of viable crown gall bacteria, may be reflected in this discrepancy. The type and moisture content of soil have been shown to affect the sorbition of methyl bromide and its efficacy as a fungicide (2, 14).

The eradication of the pathogen was not accomplished by any of the treatments included in these tests. The numbers of crown gall bacteria in treated soil will depend upon the initial population of A. tumefaciens and the amount of undecomposed gall tissue. The effectiveness of the treatment will be enhanced by a fallow period of sufficient duration to permit the breakdown of infected plant tissue and the subsequent release of the pathogenic bacteria into the soil prior to fumigation. A reduction in the numbers of the crown gall bacteria probably decreases the chance of infection when a susceptible host is planted; however, the effect of the population of the pathogen in the soil on the degree of infection was not determined.

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EFFECT OF BORDEAUX SPRAY AND PLASTIC COVERING IN PREVENTING INVASION OF LOW-TEMPERATURE-INJURED STEMS OF PRUNE TREES BY CYTOSPORA FUNGI[1]

A. W. Helton[2]

Summary

Six different treatments, consisting of various combinations of Bordeaux and protective plastic, were applied after stems of test trees had been injured with dry ice blocks.

From these experiments it was concluded that both Bordeaux sprays and plastic coverings can reduce the extent of post-freeze damage by Cytospora fungi, but that prune trees cannot be entirely protected from subsequent infection either with single applications of Bordeaux mixture or by mechanical exclusion devices such as plastic coverings. It is postulated that repeated applications of Bordeaux will not be sufficiently valuable to overcome the impractical aspects of repeated spraying during the dormant and spring periods, and it is suggested that control of the Cytospora diseases should be sought via therapeutic means.

INTRODUCTION

A cankerous disease of prune trees first was recognized as a serious problem in Idaho in 1951. Cytospora fungi were isolated consistently, and subsequent experiments demonstrated that virulent strains were of common occurrence in affected orchards (4).

During the early years of the Cytospora canker investigations, evidence was accumulated which demonstrated that, while the causal fungi most commonly invaded unthrifty trees, mechanical wounds provided adequate entry courts in vigorous trees (3). The fungi also were found to be capable of invading stems of vigorous trees that had been injured by low temperatures, and in the absence of discernible cracks in the injured surfaces (1, 2). Although specific data were not available on which to base the recommendations of a control program, growers were advised to use extreme surgery measures and to paint all wounds with a paint containing a fungicide. These measures were tried by a number of growers in the various orchard districts. The control achieved was slight and temporary.

Experiments were undertaken in which conditions following mild winter injury were simulated, and commercial Bordeaux mixture was applied at high concentration to determine its effectiveness in preventing invasion of prune trees by Cytospora fungi. Plastic envelopes were used as a mechanical inoculum-exclusion device.

MATERIALS AND METHODS

Blocks of dry ice 2 x 4 x 12 cm were prepared with a band saw. By means of a string around the center and a rubber budding strip at each end, the blocks were bound to dormant stems of 3-year-old Italian prune trees (Prunus domestica).

The trees were in a vigorous condition of growth and located on a gentle south slope. The dry ice blocks were attached to branches and trunks approximately 3 cm in diameter at the points of attachment. Paper toweling was stapled around the stems above all blocks to shade them from the direct rays of the sun.

A dense inoculum suspension of spores and mycelium was prepared from cultures of a selected Cytospora isolate (Cy-16 (3)) grown on malt agar medium at a constant temperature of 25° C. The isolate was one of known pathogenicity characteristics and one which had been obtained from a diseased Italian prune tree. The suspension was applied to certain of the frozen areas with cotton pads immediately after evaporation of the dry ice blocks.

Aside from the controls, six different treatments were employed as follows: Treatment A; tissues were frozen with dry ice blocks and the frozen areas left exposed. Treatment B; frozen areas were sprayed 4 days later with commercial Bordeaux mixture at a concentration of 25 pounds in 100 gallons of water and left exposed. Treatment C; frozen areas were covered with plastic envelopes as soon as the dry ice evaporated (cotton rings were placed around the stems above and below the frozen areas and transparent plastic sheeting bound over the rings with plastic electrician's tape to form cylinders about the frozen stems). Treatment D; frozen areas were swabbed with the inoculum suspension immediately after evaporation of the dry ice and left exposed. Treatment E; frozen areas were swabbed with the inoculum suspension as soon as the dry ice evaporated and sprayed with Bordeaux 4 days later. Treatment F; frozen areas swabbed with inoculum and covered with plastic envelopes as soon as the dry ice evaporated.

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[1] Approved by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 525.

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Reactions of test trees to the dry ice and to invading Cytospora fungi, whether from artificial or natural sources, were recorded by means of an arbitrary numerical system as follows: 0) No flat-face due to dry ice injury and no invasion by Cytospora fungi. 1) Flat-face produced at the point of attachment of the dry ice block (by subsequent growth of adjacent stem tissues) and normal development of wound callus around the injured and/or invaded attachment point. 2) Flat-face with no appreciable marginal callus. 3) Flat-face lightly pimpled with Cytospora pycnidial pustules. 4) Flat-face moderately pimpled. 5) Flat-face densely pimpled. 6) Exudation of gum from the flat-face and/or adjacent areas. 7) An actively expanding Cytospora canker at the flat-face. 8) Flagging of parts terminal to the infected flat-face, that is, foliage discolored and/or drooping. 9) All parts of the stem above the infected flat-face dead. 10) Canker expanding downward from the infected flat-face. 11) Infected tree dead.

All tests were replicated nine times.

RESULTS

The average evaporation time of the dry ice blocks was 2 hours. The average air temperature in the plot during the evaporation period was 26° C.

Unfrozen control trees developed no symptoms. Unfrozen control trees swabbed with the inoculum suspension also failed to develop symptoms.

Since the results of the six treatments are recorded as averages of nine replicates, the individual cases of virulent infection are not clearly revealed in Figure 1. Two such cases occurred in treatment A, one in D, one in E, one in F, and none in B and C. The infections were not considered virulent unless cankers still were actively expanding, or infected trees were dead, 420 days after freezing of the stems with the dry ice blocks on May 1, 1958.

A comparison of the spraying results of treatments B and E (Fig. 1) shows that while natural inoculum caused earlier damage (B), the effect of the applied inoculum (E) was more sustained. Similarly, inoculation before covering with plastic (F), resulted both in more severe and more sustained Cytospora damage than occurred when the artificial inoculum was omitted (C).

The plastic did not exclude all inoculum from natural sources, and the Bordeaux spray either did not destroy all artificially applied inoculum or did not protect the frozen areas after spring growth opened fissures in the Bordeaux covering. As Figure 1 (treatments B and C) shows, however, both spraying and covering with plastic reduced the incidence of infection unless artificial inoculations were made before the spray or plastic was applied.

DISCUSSION AND CONCLUSIONS

The trees were sprayed 4 days after freezing of the stems to allow inoculum from natural sources (as well as the applied suspension) to accumulate on the frozen tissues and because such a time lag between injury and spraying would be likely to occur in commercial orchards.

In treatment A, where stems were frozen and left exposed, natural infection produced more damaging results than where the inoculum suspension had been applied directly to the injured surfaces. This demonstrated that inoculum was omnipresent in the environment even though the location was not in an orcharding area, and suggested that strains of the fungi were present in the environment which were more virulent than the applied inoculum.

The presence of infection in treatment B (where the frozen areas were sprayed with Bordeaux mixture after 4 days) and in treatment C (where plastic was applied to exclude natural inoculum) suggests that neither spraying nor mechanical methods of exclusion can be expected to prevent infection completely under the conditions of the tests, and particularly not in an infested orchard environment.

Cytospora invasion of the trees reached most prominent proportions in treatment D, which indicated that the artificially applied inoculum was having at least a slight effect and perhaps was acting additively with the natural inoculum to some degree. This fits in well with past experience in Idaho wherein various strains have been isolated from the same canker area on an infected tree.

While the data indicate that either strong concentrations of Bordeaux mixture or plastic coverings can reduce Cytospora invasion if applied to prune trees within 4 days after severe freezes, the data also show that these measures cannot be expected to prevent infection entirely. Repeated applications of Bordeaux during the dormant or delayed-dormant stages perhaps would yield better results, but such a procedure would be prohibitively expensive if not otherwise impossible from the practical standpoint.

Data reported thus far, therefore, indicate that the Cytospora canker disease will continue to be a troublesome problem in orchards where even moderate low-temperature injury occasionally occurs unless better methods of inoculum-exclusion are found. Where surgery, surgery plus fungicidal paints, strong dormant-period sprays, and physical methods of inoculum exclusion do not yield satisfactory control, other methods are needed. It is postulated that the Cytospora disease, and other deeply penetrating pathogens of fruit trees, will be reduced to minor proportions only through use of systemic, and host-compatible chemicals.

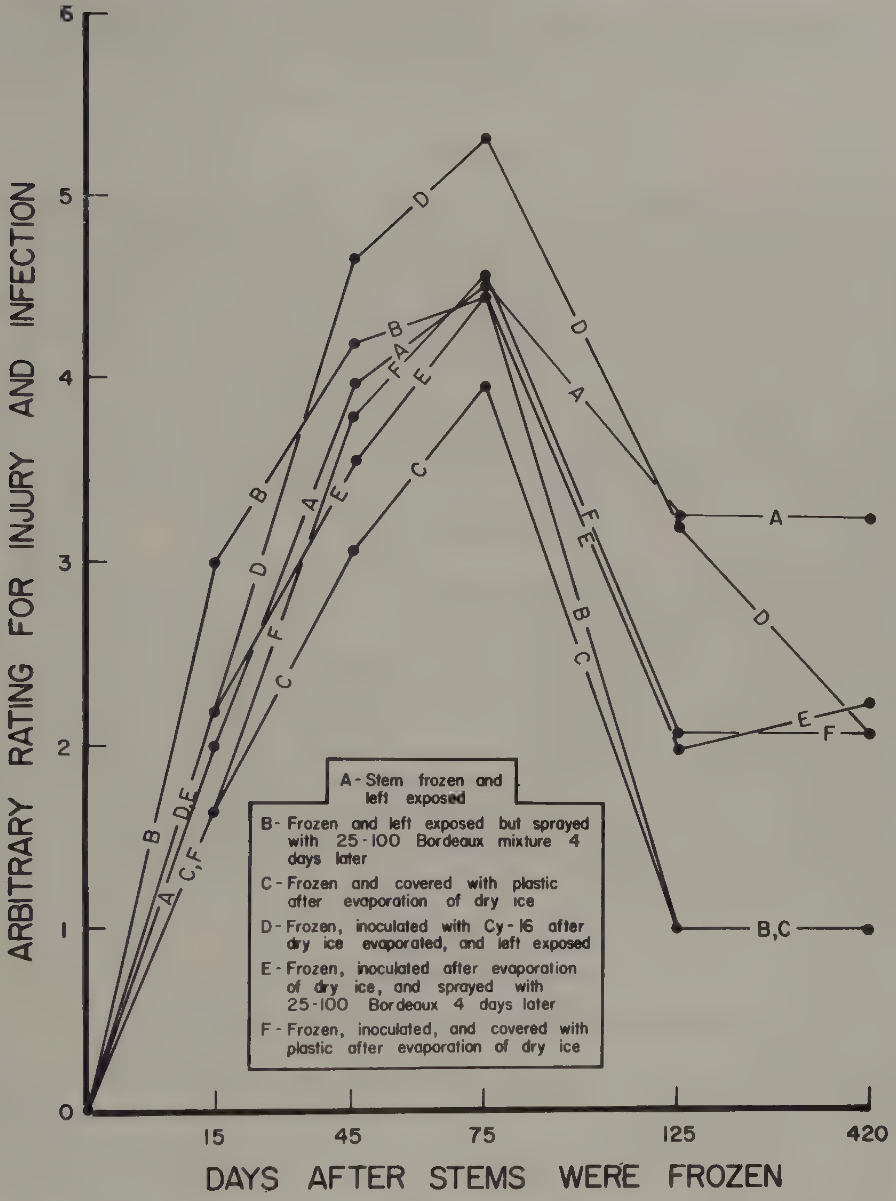

FIGURE 1. Degree of low temperature injury and effect of Bordeaux spray and plastic covering on development of Cytospora canker disease when stem tissues of dormant 3-year-old Italian prune trees were injured locally with attached dry ice blocks prior to swabbing-on of inoculum. Each value is the average of nine replicates.

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A COMPARISON OF APPLE VIRUS INOCULUM FROM STEM PITTED, DAPPLE APPLE, FLAT LIMB, AND MOSAIC INFECTED TREES

R. C. McCrum and M. T. Hilborn[1]

Abstract

Comparisons between apple virus "isolates" based on indicator plant symptoms in field tests imply that the virus factor responsible for stem pitting in Virginia Crab is not related to virus entities producing mosaic, dapple apple, chlorotic leaf spot or rubbery wood symptoms in apple. These data suggest that a possible relationship exists between stem pitting and flat limb in regard to the causal agent. Wood pitting of the Russian apple clone R-12740-7A was caused by dapple apple inoculum which did not pit Virginia Crab. This difference in pitting as a host reaction indicates either separate virus strains of stem pitting capacity, or similar symptoms by distinctly different viruses. It would seem that the term stem pitting should be restricted to the symptom reaction produced on Virginia Crab and that Virginia Crab be designated as a standard indicator for the apple stem pitting virus.

INTRODUCTION

Although many new virus disorders of apple have been reported recently (5), little information is available concerning the relationships between these disorders. Working with such indicators as Lord Lambourne, Virginia Crab, and Prunus tomentosa, Welsh and Keane (12) present "limited but strong evidence" that the virus factors for rubbery wood and stem pitting symptoms are distinct. Although a high frequency of association existed in their indexing tests, Mink and Shay (8) published evidence to suggest that chlorotic leaf spot and stem pitting are caused by separate entities. As a result of index tests conducted on a 5-year-old orchard, Cation (3) found that chlorotic leaf spot and stem pitting disorders appeared to be caused by different viruses since they were not always found in association. Cation (2) also found that isolates of dwarf fruit and Spy 227-lethal, containing stem pitting and chlorotic leaf spot factors, did not produce rubbery wood or mosaic symptoms when budded to Lord Lambourne. Barrat, et al. (1) state that the virus factor for stem pitting and for dapple apple occur independently of each other.

METHODS

Virus inoculum used in this study consisted of apple budwood from orchard trees displaying the following symptoms:

1. Dapple Apple: Obtained from W. W. Smith, Department of Horticulture, University of New Hampshire. The budwood was taken from Cortland scions top-worked to a non-pitted Virginia Crab framework tree (1).
2. Flat Limb: Obtained from J. F. Hockey's experimental plot of Gravenstein trees at Kentville, Nova Scotia (4).
3. Stem Pitting: Obtained from budwood of Baldwin top-worked to a pitted Virginia Crab framework tree located in a hardy stock planting at the Maine Agricultural Experiment Station Farm, Monmouth, Maine.
4. Mosaic: Obtained from a standard Cortland apple tree in an orchard at Kents Hill, Maine.

Apple varieties used as indicators included: The Russian apple clone R-12740-7A which develops a characteristic leaf spot reaction designated chlorotic leaf spot (8); Lord Lambourne as a selective indicator for rubbery wood and for its sensitivity to apple mosaic strains; and Virginia Crab as an indicator for stem pitting. Other crab apple varieties have been suggested as indicators for stem pitting (6, 7, 8, 9, 11); however, Virginia Crab was selected as this variety has been widely used and is most commonly associated with the stem pitting disorder.

In the summers of 1959 and 1960 buds from infected trees were inserted into apple seedlings directly below buds of the indicator varieties in a double budding method described by Posnette and Cropley (10). With the Russian indicator R-12740-7A however, 1- and 2-year-old shoots were inoculated directly as bud take of this variety is frequently low when both the indicator R-12740-7A and the bud to be indexed are inserted at the same time. Apparently the R-12740-7A is extremely sensitive to latent viruses contained in commercial scion varieties. The double budded apple seedlings were cut back to the indicator buds the spring following budding, and readings were taken in 1961.

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RESULTS

Readings based on symptoms expressed by the developing indicator shoots are shown in Table 1. Stem pitting inoculum did not produce rubbery wood or mosaic symptoms in two clones of Lord Lambourne over a period of 2 years. Subsequent indexing showed that the Lord Lambourne clones reported in Table 1 contained the stem pitting factor. Stem pitting inoculum produced chlorotic leaf spot and severe dieback (Fig. 1) as well as wood pitting in the Russian R-12740-7A indicator. It also caused pitting in Virginia Crab.

Table 1. Reactions of three indicators to apple virus isolates.

	Indicators		
Isolates	Virginia Crab	R-12740-7A	Lord Lambourne
Stem pitting	pitting 5-5/5[a]	pitting 5-5/5	rubbery wood 0-5/5 0-4/5
		dieback 5-5/5	mosaic 0-5/5 0-4/5
		chlorotic leaf spot 5-5/5	
Flat limb	pitting 5-5/5	pitting 5-3/5	rubbery wood 0-3/5
	mosaic 2-5/5	dieback 3-3/5	mosaic 0-3/5
		chlorotic leaf spot 5-3/5	
Dapple apple	pitting 0-2/5	pitting 5-5/5	
		dieback 0-5/5	
		chlorotic leaf spot 5-5/5	
Mosaic	pitting 4-4/4	pitting 5-5/5	rubbery wood 0-6/8
	mosaic 4-4/4	dieback 5-5/5	mosaic 6-6/8
		chlorotic leaf spot 5-5/5	

[a] Number of budlings showing symptoms -- number of buds united/number of buds inserted.

Budwood from flat limb infected Gravenstein induced stem pitting in Virginia Crab. Such inoculum did not cause rubbery wood symptoms in 1-year-old shoots of Lord Lambourne, nor did it produce mosaic symptoms on these same shoots. This flat limb source did, however, produce mosaic reactions in two of the inoculated Virginia Crab indicators. Why mosaic reactions occurred in Virginia Crab and not in Lord Lambourne can not at present be explained.

Pitting and chlorotic leaf spot occurred in all Russian R-12740-7A indicators; and severe dieback occurred where bud take of the flat limb isolate was successful. However, the two R-12740-7A indicators, which did not show dieback symptoms, developed chlorotic leaf spot and wood pitting reactions. Evidently the dieback factor was not transmitted due to bud failure, although factors for pitting and chlorotic leaf spot were transmitted through the initial bud union. The syndrome complex of these two indicators was identical to that produced by the dapple apple inoculum which was not carrying the Virginia Crab stem pitting entity.

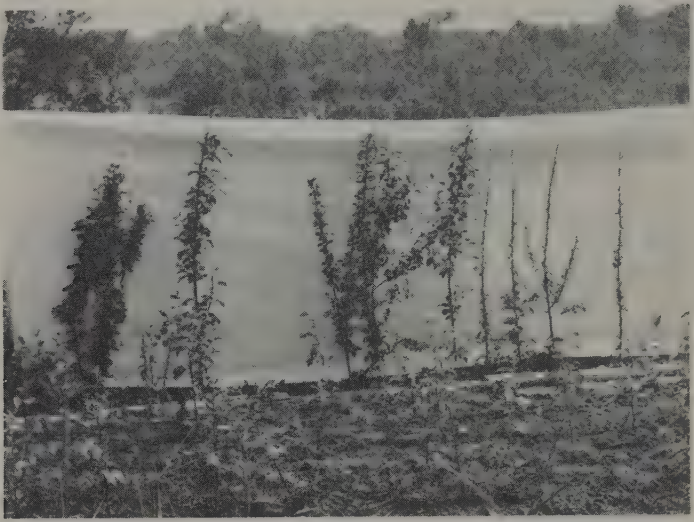

FIGURE 1. Russian apple clone R-12740-7A. Five trees on left inoculated with dapple apple. Four trees on right inoculated with stem pitting. Note dieback in trees inoculated with stem pitting.

As expected, dapple apple inoculum did not pit Virginia Crab since the original material came from top-worked non-pitted Virginia Crab trees. It did cause chlorotic leaf spot symptoms in the Russian R-12740-7A indicator as well as wood pitting. However, no dieback was observed (Fig. 1) and growth of the Russian clone with the dapple apple inoculum was superior to growth of the same clone inoculated with the other virus isolates, with the exception of the two mentioned in the flat limb series.

Mosaic inoculum from the Cortland variety carried the factor for stem pitting as shown in Virginia Crab. It also produced mosaic in all inoculated Lord Lambourne and Virginia Crab shoots. It did not cause rubbery wood symptoms in 1-year-old shoots of Lord Lambourne. The Russian clone R-12740-7A developed chlorotic leaf spot accompanied by severe dieback (Fig. 2) and wood pitting when inoculated with mosaic infected material.

DISCUSSION

Symptom reactions of Lord Lambourne, Virginia Crab and the Russian apple clone R-12740-7A to inoculum from mosaic, stem pitted, flat limb, and dapple apple infected trees indicate several differences in regard to host specificity.

The virus factor for stem pitting occurred independently of virus entities responsible for mosaic, chlorotic leaf spot and rubbery wood disorders. The severe dieback that occurred in the Russian R-12740-7A indicator as a distinct response may have been due to stem pitting, since dieback occurred with all inoculum sources tested with the exception of dapple apple inoculum which did not contain stem pitting.

Flat limb and stem pitting may be manifestations of a common causal agent. Further testing, however, will be necessary to segregate, if possible, various factors contained in this flat limb isolate as well as to cross inoculate stem pitting material back to Gravenstein apple to test this hypothesis.

Although the dapple apple isolate did not cause stem pitting in Virginia Crab, virus contained in this bud source did produce wood pitting in the Russian R-12740-7A indicator. This latter fact indicates that not all wood pitting of apple virus origin is due to a single virus entity.

This observation was also made by Mink and Shay (8), who found that inoculum from a larger proportion of apple varieties induced pitting on the Russian apple clones 45-39 and 69-105 as compared with Virginia Crab. In fact, of 36 varieties indexed, 15 gave negative reactions on Virginia Crab while budwood from all 36 varieties induced pitting on one or both of the Russian clones. Such data suggest that the term "stem pitting" should be restricted to that which occurs on the indicator variety Virginia Crab.

Attempts to classify causal agents of apple virus disorders is complicated by the fact that present commercial varieties can and do contain several pathogenic virus entities, many of which may exist in a latent form. Until these factors can be segregated into single entities, present and future classification based on similar symptoms occurring in different indicators is of dubious value. It is suggested that standard indicators, preferably apple varieties in which the disorder was first reported, be used to report new records of apple disorders.

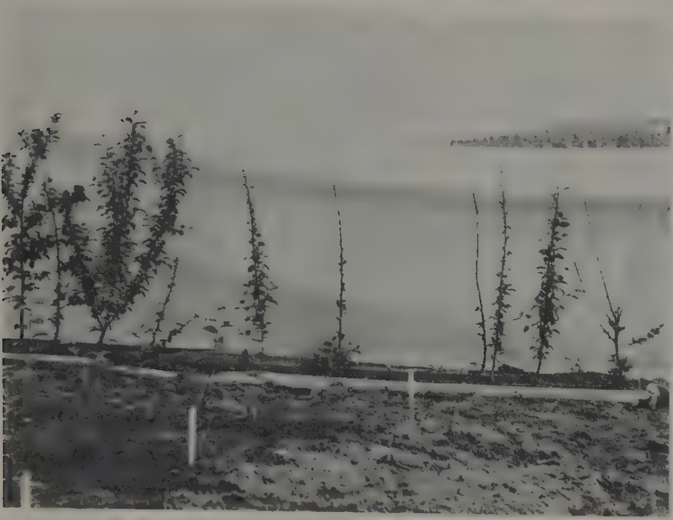

FIGURE 2. Russian apple clone R-12740-7A. Five trees on left inoculated with flat limb. Five trees on right inoculated with mosaic. Note dieback on 1-and 2-year-old trees inoculated with mosaic and in two of the trees inoculated with flat limb.

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MAINE AGRICULTURAL EXPERIMENT STATION, ORONO

NONPATHOGENICITY OF PRATYLENCHUS PENETRANS TO STERILE LOW-BUSH BLUEBERRY SEEDLINGS

R. C. McCrum and M. T. Hilborn

During the course of a study on the relationship of mycorrhizal fungi to the growth of the low-bush blueberry plant an opportunity arose to investigate the pathogenicity in pure culture of Pratylenchus penetrans to wild low-bush blueberries. A recently published survey had recorded 19 genera of stylet-bearing nematodes as being associated with the soil and roots of wild blueberry plants[1]. Since the mycorrhizal study involved the use of sterile cultures of blueberry plants it was thought that inoculation of these plants with cultures of Pratylenchus penetrans would offer a direct approach to a study of nematode pathogenicity.

METHODS

Blueberry seeds were obtained from fresh, frozen, wild low-bush blueberries processed in August 1960. On November 10, 1960 frozen berries from the above pack were surface sterilized in a 1:10 Clorox dip for 20 minutes and the seeds removed. These seeds were then put into 20-mm test tubes on the surface of an agar medium for germination. Each tube contained 15 cc of a 5% agar medium containing White's standard mineral salts with the omission of iron and the organic complexes. On December 5, 1960 a total of 30 tubes containing germinated blueberry seeds were inoculated with small pieces of sterile corn roots infested with P. penetrans. The original nematode culture was obtained through the courtesy of Dr. W. R. Jenkins, Rutgers University and had been maintained on sterile corn roots. Tubes so inoculated were placed by a laboratory window with the agar portion of the tube wrapped in kraft paper sleeves to prevent indirect illumination of the roots and to allow visual inspection of the developing blueberry plant.

RESULTS

Two days after inoculation, nematodes were seen in 19 of the tubes colonizing around the young developing roots (Fig. 1). Up to December 19, 1960, two weeks after the initial inoculation, the number of nematodes per tube increased due to their emergence from the corn root piece used as original inoculum. A few eggs were also seen around the developing blueberry roots originating from gravid females in the corn root inoculum. Although nematodes were observed in close association with the developing blueberry root tips no necrosis of root tissue was observed nor was any direct penetration of root cells recorded. In a few tubes an actinomycete type fungus occurred surrounding the rhizosphere and in some cases the nematodes were in close association with this material (Fig. 2). No reproduction of P. penetrans occurred and a gradual reduction of viable nematodes was noted. Some tubes supported viable nematodes 70 days from the initial inoculation.

Measurements of seedling growth were made 80 days after inoculation. Seedling growth in 15 tubes containing nematodes was compared with an equal number of tubes without P. penetrans (Table 1). The number of nematodes per tube varied from 4 to over 40.

DISCUSSION

Although P. penetrans was seen to colonize around blueberry root tips and advance downward with the growing root tips no visible damage to the roots occurred. Blueberry seedlings growing in test tubes containing the nematode did not exhibit any reduction in growth as compared with uninoculated seedlings. In fact, seedlings containing nematode cultures developed greater shoot growth and contained more nodes per shoot than did the uninoculated checks. Differences in shoot growth could have been the result of added nutrients supplied by the corn root pieces used as inoculum since the uninoculated seedlings did not receive this material. The increase in growth of blueberries in tubes containing nematodes might also have resulted from the additional mineral or organic constituents released by the nematodes themselves. Availability of nutrients to the roots might have been enhanced by the physical action of the nematodes. In no case was infection or penetration of blueberry roots observed under the conditions of this test.

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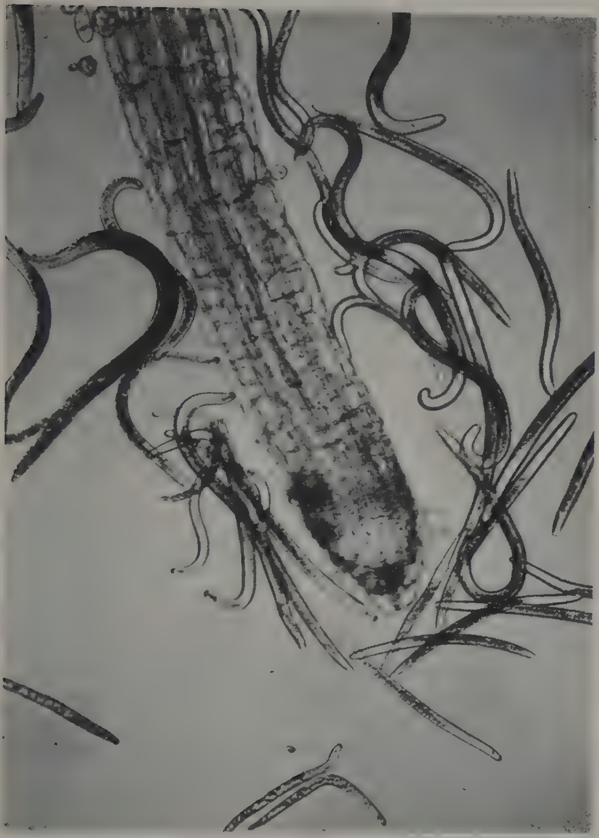

FIGURE 1. Pratylenchus penetrans clustering around a growing root of the low-bush blueberry.

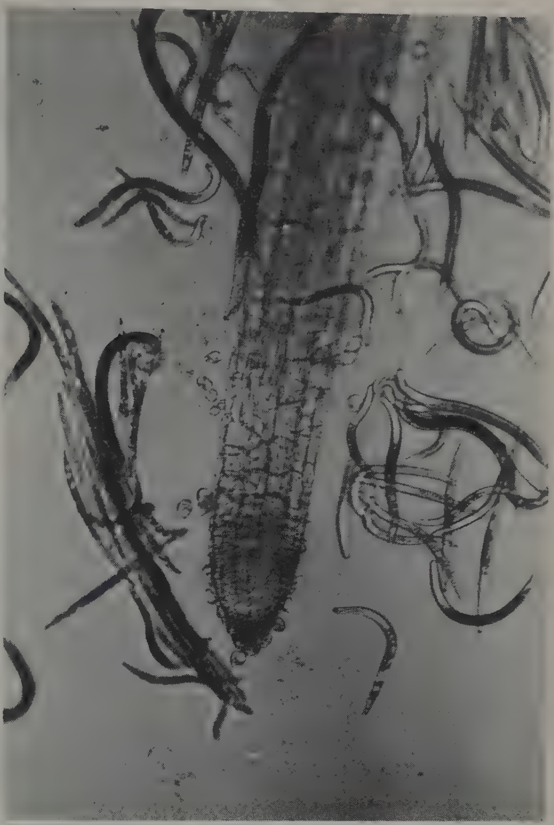

FIGURE 2. Pratylenchus penetrans apparently feeding on an actinomycete type fungus growing in association with a low-bush blueberry root.

Table 1. Combined root and shoot growth of blueberry seedlings.

	Inoculated with P. penetrans		Non-inoculated	
Seedling no.	length in mm	no. of nodes	length in mm	no. of nodes
1	9	2	10	3
2	16	4	12	4
3	22	7	12	4
4	15	4	9	2
5	8	2	13	2
6	20	6	10	2
7	15	2	9	1
8	16	5	12	3
9	8	3	9	2
10	16	5	9	1
11	15	6	8	1
12	15	6	9	1
13	26	9	10	1
14	20	5	11	1
15	13	3	9	1
Average	15.6	4.6	10.1	1.9

MAINE AGRICULTURAL EXPERIMENT STATION, ORONO

ROOT GRAFTING OF CITY-PLANTED AMERICAN ELMS

E. B. Himelick and Dan Neely[1]

There is still considerable speculation on the importance of root grafts as a means for local spread of Dutch elm disease even after 30 years of intensive research on this disease. The movement of the Dutch elm disease fungus from tree to tree through root grafts has not been emphasized by research people carrying on studies for control of the disease. Spread of the fungus through the root systems from diseased to healthy trees is not prevented by conventional control measures of sanitation and spraying. In communities conducting intensive Dutch elm disease control programs, the authors have observed the spread of the disease from affected to nearby healthy trees when transmission by the American and European elm bark beetles was not believed probable. In such cases, the original diseased trees were removed and burned prior to development and emergence of the beetles.

The studies of Verrall and Graham (6) indicate that the Dutch elm disease fungus can readily pass through natural root unions. It was also shown that a common root system frequently occurs in elm stands where trees are 8 inches dbh or larger and 7 feet or less apart. Root grafting was observed between trees that were up to 17 feet apart. The use of dyes and poisons to locate and demonstrate the presence of root grafts between oaks has been reported by several workers (4, 5). After one tree of a group had been chemically treated, injury typical of the material used was often evident on neighboring trees. The existence of root grafts between treated trees and trees showing chemical injury was definitely established by removing the soil from their roots. Continuous discoloration was present in the roots from the treated trees to adjacent trees.

There are reports of "backflash" resulting from the use of silvicides in forest management (1, 2). Backflash is the movement of poison through root grafts from poisoned to unpoisoned trees. The phenomenon of backflash is the basis for this report on root grafting of city-planted American elms.

The city of Batavia, Illinois has conducted a municipal Dutch elm disease control program for 6 years. In the spring of 1961, a new procedure was included in the program. This procedure consisted of poisoning all diseased elms with the herbicide Atlas D[2] at the time of early symptom expression. The herbicide was applied near the base of the trunk in ax frills. The frills were sprayed with creosote and covered with a heavy roofing paper. The method used by the treating crew was similar to that previously described by the authors (3).

On September 14, before fall leaf coloration was apparent, the authors made a survey of all the elms in Batavia. At this time a large number of elms adjacent to diseased elms treated with Atlas D showed symptoms typical of herbicide injury. This injury appeared as browning of leaves on one to several branches. Usually only one or two branches on a tree showed injury. Rarely was the whole crown of a tree affected. In most instances the injured branches on the untreated trees were on the side nearest the treated trees.

In previous studies we have observed that Atlas D herbicide is translocated downward from ax frills into the roots of treated elms. In trees treated in Batavia, the herbicide was translocated from the point of application in sufficient concentration through grafted roots to injure or kill portions of neighboring elms. Discoloration of the cambium and xylem typical of Atlas D injury was observed in the trunks and buttress roots of adjacent untreated elms having foliage injury. The herbicide moved laterally only to a very limited extent and only branches above buttress roots with internal discoloration were killed in most instances.

The data collected from the survey of elms in Batavia are given in Table 1. A total of 155 treated elms was tallied during the survey; however, only 88 of these treated trees were within 40 feet of untreated trees. The table lists only those untreated trees that were within 40 feet of trees poisoned with Atlas D during the summer of 1961. Occasionally more than one untreated tree was growing within 40 feet of a treated tree. Each untreated tree was recorded as a separate instance of possible root grafting. Our results indicate that 43% of the elms closer than 16 feet are root grafted and that 29% of the elms closer than 26 feet are root grafted. Although the diameters of both treated and neighboring untreated trees were recorded, we found no significant relationship between the size of trees and distances between trees, and the amount of root grafting that occurred. The elms varied from 10 to 36 inches dbh. The treated trees averaged

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[1] Associate Plant Pathologists, Illinois Natural History Survey, Urbana, Illinois.

[2] Atlas D, formulated by the Chipman Chemical Company, contains 40% active sodium arsenite, a blue dye, and an animal repellant.

Table 1. Root grafting between untreated and treated elms as indicated by backflash resulting from the use of Atlas D herbicide.

Distance in feet between treated and untreated elms	Number of untreated elms observed	Untreated elms grafted to treated elms	
		number	%
5-10	9	3	33
11-15	14	7	50
16-20	29	6	21
21-25	20	5	25
26-30	13	-	-
31-35	2	-	-
36-40	1	-	-
	88	21	23.8

22.2 inches dbh and the untreated trees averaged 20.7 inches dbh. Spacings of 20, 25, and 30 feet had been used in the city planting, with the 20-foot spacing being quite common.

From these data it was concluded that root grafting of American elms growing in parkways or on private grounds occurs for distances up to 25 feet between trees. In Batavia, 24% of the healthy elms growing within 40 feet of elms affected with Dutch elm disease were connected with the diseased trees by natural root graft unions. This high occurrence of root grafting appears to be related to the close spacing of trees and emphasizes the importance of spacing street trees at a distance greater than 30 feet.

Cities with Dutch elm disease control programs should consider the value of trenching between diseased and healthy trees when the distance between trees is less than 30 feet. Trenching to a depth of 30 inches with a spade or with a small trenching machine will break most root grafts between elms.

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ON THE ATTEMPTED INDUCTION OF RESISTANCE IN OATS TO CROWN RUST RACE 264 BY ARTIFICIAL IRRADIATION[1]

M. D. Simons, R. S. Caldecott, and K. J. Frey[2]

Summary

Numerous reports of the successful induction of disease resistance in cereals by artificial irradiation prompted attempts to induce resistance to the highly aggressive oat crown rust race 264. In work extending over several years in which various dosages of different types of irradiation were applied to seeds of five varieties of hexaploid oats, a total of about 400,000 seedlings were tested for disease reaction. Chlorophyll mutants were much more common in the irradiated material than in nontreated check material. Putative mutations observed for seedling resistance to several common races of crown rust, for field resistance to common races of crown rust, and for resistance to Victoria blight could not be shown conclusively to result from irradiation. No mutations for seedling resistance to crown rust race 264 were observed.

Most studies in which irradiation was used to induce disease resistance in crop plants have been undertaken on an exploratory basis with the primary objective of determining whether resistance could be induced. The number of affirmative reports leaves little doubt that disease reaction can be altered by irradiation. Very few of these studies, however, have induced useful, new genes. This is not surprising since they were designed primarily to demonstrate a principle rather than to solve a specific problem.

The investigation described here was an attempt to use artificial irradiation in the solution of a practical problem, namely, the induction of seedling resistance to crown rust (Puccinia coronata Cda. var. avenae Fraser & Led.) race 264 in hexaploid oats. Since extensive testing has failed to reveal such resistance in the hexaploid oats of the World Collection (23), the successful induction of mutations that would condition resistance would be a significant contribution from the practical standpoint and serve as strong confirmation of previous reports of artificial induction of genes for disease resistance.

REVIEW OF LITERATURE

Literature on the general topic of the genetic effects of irradiation on plants has been reviewed by Konzak (13). This review will be confined to pertinent literature dealing with selected aspects of the effects of radiation on resistance of small grains to obligately-parasitic fungi.

As early as 1942, Freisleben and Lein (7) reported induction of disease resistance in cereals by irradiation. They applied X-rays (12,000r) to barley seed of a variety susceptible to powdery mildew (Erysiphe graminis DC. f. sp. hordei Em. Marchal). A mutant line resistant to powdery mildew was obtained. Another report of X-ray-induced resistance to this disease was published in 1951 (1). Further study of this resistant material (18) strongly indicated that resistance genes had been produced that differed from any of those known in previously existing sources of resistance. These studies also showed that induced genes for resistance need not be recessive. The work of Favret and Rodriguez (5) provided another example of an induced, dominant mutation for mildew resistance in barley.

Hansel and Zakovsky (9) tested 150,000 X_4 barley plants for reaction to a mixture of races of powdery mildew and found 190 with some degree of resistance under greenhouse conditions. Seventy-two of these were resistant in the field. This high rate of mutation was similar to that observed for albinism and was attributed to the presence of a large number of loci rather than to a high degree of mutability in a few loci. In contrast, Davies and Wall (4) found no mildew-resistant barley plants among 18,000 R_2 seedlings (gamma rays or thermal neutrons), whereas 141 chlorophyll mutants did appear.

The induction of barley stem rust resistance by irradiation has also been reported (20).

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[1] Cooperative investigation of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Iowa Agricultural and Home Economics Experiment Station (Journal Paper No. J-4243, Project No. 1176), Ames, Iowa.

[2] Respectively, Pathologist and Geneticist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Professor of Agronomy, Iowa State University.

A number of investigators (8,10,17, and 24) reported a high rate of irradiation-induced mutations for resistance to stem rust of oats (Puccinia graminis Pers. f. sp. avenae Eriks. & E. Henn.). Caldecott, Stevens, and Roberts (2), however, found no resistance to stem rust in oats derived from irradiated material when field hybridization was prevented. Numerous resistant plants occurred in corresponding material that had been planted in proximity to other resistant oats. This suggests that some of the stem rust resistance attributed to mutation may have resulted from outcrossing. Konzak (14) also questioned the validity of some earlier reports of induced stem rust resistance in oats. He did, however, find a mutant in irradiated Clintafe oats that showed a previously unknown temperature-sensitive response for reaction to race 7A. Irradiation-induced resistance to crown rust and to Victoria blight, caused by Cochliobolus victoriae Nelson (Helminthosporium victoriae Meehan & Murphy), has also been reported (12). Chapman, et al. (3) treated 3,000 seeds of Floriland oats with thermal neutrons. Certain lines derived from this material possessed good field resistance to crown rust race 264, and others exhibited a mesothetic reaction to this race in the greenhouse. However, no seedling resistance was obtained.

The results of irradiation of wheat for induction of disease resistance are in general agreement with those for barley and oats. Konzak (11) reported that 4% and 2% of the lines of Lerma wheat derived from X-ray and thermal neutron treatment, respectively, contained mutants for resistance to stem rust (Puccinia graminis Pers. f. sp. tritici Eriks. & E. Henn.). Myers, et al. (17) and MacKey (16) also reported obtaining wheat stem rust resistance from irradiation. Konzak, et al. (15) found that one N_3 line bred true for resistance to stripe rust (Puccinia striiformis West.) and was otherwise typical of Gabo, the parental variety. Twelve additional progenies segregated for various degrees of resistance.

METHODS AND RESULTS

This study consisted of several phases. In the first, 300 seeds from a pure stock of each of three oat varieties (Park, C.I. 6611; C.I. 6700; and Mo. 0-205, C.I. 4988) and 200 of Simcoe (C.I. 6767) were treated with X-rays (20,000r). The seed of each variety was grown in a separate room of the greenhouse to minimize the possibility of crossing, and the seed produced on the resulting plants was space-planted in the field. A total of 20 X_3 seedlings from each of 4200 X_2 plants representing over 500 irradiated seeds were tested. Lines derived from Park, which carries the Victoria gene or gene complex, were tested for reaction to C. victoriae (Table 1). Lines from C.I. 6700, which has the Landhafer gene for crown rust reaction, were tested for reaction to race 263. Lines from Mo. 0-205 and from Simcoe were tested with race 202.

Table 1. Reactions of certain oat varieties to five races of the crown rust fungus and to C. victoriae.

	Crown rust race					
Variety	202	216	263	264	290	C. victoriae
C.I. 6700	Res.	Res.	Susc.	Susc.	Susc.	Res.
Clintland	Res.	Res.	Susc.	Susc.	Susc.	Res.
Park	Res.	Susc.	Res.	Susc.	Res.	Susc.
Mo. 0-205	Susc.	Susc.	Susc.	Susc.	Res.	Res.
Simcoe	Susc.	Susc.	Susc.	Susc.	Susc.	Res.

No resistant plants were found in materials derived from C.I. 6700 or Simcoe. Three of 810 lines from irradiated Park segregated for resistance to C. victoriae, and four additional lines were homozygous resistant. This frequency of resistant progenies was comparable to that reported by Konzak (12). All plants resistant to C. victoriae, however, were susceptible to crown rust race 202. Since these lines had no practical value, they were discarded at this point.

Four of the 1629 lines of Mo. 0-205 appeared to be homozygous resistant to race 202. These lines all proved to be susceptible to race 264. Further pathological and genetic studies showed that these lines contained a gene for resistance at the same locus as the Landhafer gene. The degree of resistance afforded by the Landhafer gene can be modified by temperature and level of nitrogen (21). When the mutant lines were tested along with varieties containing the Landhafer gene for reaction to various races of crown rust under varying environmental conditions, no consistent, measurable difference was found between the reactions of the "mutants" and varieties with the Landhafer gene.

The failure of the first phase of the study to reveal any new and useful genes for crown rust resistance may have been due to the relatively small number of seeds irradiated. The variety Mo. 0-205 was chosen for the second phase because it was known to have a higher mutation rate than other varieties. About 110,000 seeds of this variety were treated with thermal neutron irradiation at the Brookhaven National Laboratory. The irradiated seed was grown and harvested in bulk. About 150,000 seedlings from this N_2 bulked material were tested for reaction to crown rust race 264. Initially some seedlings showed minor differences in reaction. Further study showed that this was incorrect and the final conclusion was that not one of the 150,000 seedlings differed significantly from Mo. 0-205 in reaction to race 264.

Only one chlorophyll mutant was found among 45,000 seedlings of the nontreated Mo. 0-205, whereas 53 chlorophyll mutants occurred in the 150,000 seedlings derived from irradiated material. This showed that sufficient irradiation had been applied to induce mutations.

In the third phase of the study, material recurrently irradiated in succeeding generations, originally treated for another purpose, was used. A total of 166,000 seedlings, 100 from each of 1660 lines derived from Mo. 0-205, Clintland, and Park varieties were tested for reaction to race 264. Each line showed a visible mutation of some kind as a result of the treatment, verifying that an appreciable amount of irradiation had been received. Some plants with an intermediate reaction appeared in lines from Mo. 0-205 and Clintland. Subsequent testing of these plants, however, showed that none consistently exhibited seedling resistance.

All plants that originally appeared to have any degree of resistance were grown to maturity. Seed from them was planted in a field crown rust nursery. Two lines, both derived from Mo. 0-205, contained plants ranging in reaction from susceptible to resistant. In the following year, head selections from these plants were again grown in the field and subjected to an epiphytotic of crown rust races 203, 216, and 290. A considerable range in reaction type was again apparent, but several of the head selections, all from the same original line, appeared to be breeding true for a high type of field resistance. Unfortunately, the isolation of these lines does not constitute conclusive evidence of the induction of crown rust resistance by irradiation. Many varieties with a similar type of field resistance are known, and the possibility of an accidental outcross to one of them cannot be disproven. Genetic studies to prove that these selections differ from all previously known field-resistant oat strains would not be feasible.

DISCUSSION

The failure of the present study to achieve its objective, namely, to isolate induced mutations for resistance to crown rust race 264, was disappointing in light of the successes reported in the literature. A possible explanation of this failure is that certain gene mutations occur with a lower frequency than others. The majority of the mutations for disease resistance in the small grains have been genes that were already known. Logically, then, genes that have arisen naturally may be more easily produced artificially than some hypothetical gene that has never been found.

There is also a possibility that specific types of gene action cannot occur in particular genotypes. A single gene, for example, conferring immunity from all oat diseases could hardly be hoped for because of the wide range of biochemical and physiological requirements of different pathogens. Although the existence of large numbers of host genes for resistance and parasite genes for virulence argues against such a possibility in the case of a hypothetical gene for resistance to crown rust race 264, this explanation is attractive for hexaploid oats. However, genes for resistance to race 264 are known in diploid and tetraploid oats (22). The transfer of one such gene into a hexaploid oat strain (19) indicates that these genes can function efficiently within the genetic background of hexaploid oats.

Flor's (6) demonstration of a "gene for gene" relationship between Melampsora lini (Ehrenb.) Lév. and its host suggests that a locus will be found in cultivated oats that is associated with reaction to crown rust race 264.

When taken together, these considerations indicate that there is a good possibility that a gene for resistance to race 264 may be induced or may occur spontaneously. On the other hand, these studies suggest that the rate of mutation for the locus conditioning resistance is very low. Consequently, large populations of irradiated material will have to be screened before there is a good probability of obtaining a resistant variant.

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ROOT-LESION NEMATODES IN ENGLISH HOLLY (*ILEX AQUIFOLIUM*) IN BRITISH COLUMBIA[1]

J. E. Bosher and W. R. Orchard

Recent interest in the commercial production of English holly (*Ilex aquifolium*) in the southern coastal regions of British Columbia has led to an evaluation of the reasons for plant decline observed in several of the orchards on Vancouver Island.

The occurrence of a species of *Paratylenchus* in the root zone of holly associated with a decline in vigor was recorded in 1954[2]. Subsequent observations resulted in the frequent discovery of species of *Criconema*, *Criconemoides*, *Paratylenchus*, *Pratylenchus*, and *Xiphinema* in association with root degeneration. Results of a survey in May-June 1961 in 10 orchards recorded determinations of nematode populations in the roots and root zone of trees selected on the basis of various decline symptoms as compared with selected healthy trees. Decline symptoms were classified as "poor" when trees carried many dead terminal twigs and sparse foliage; "pale" indicated trees with pale-green lustreless foliage; "bronzed" referred to trees with yellow or bronze color of the leaves; "leaf drop" indicated extensive premature dehiscence of 2-year-old leaves. Results are shown in Tables 1 and 2.

Data in the tables show that positive correlation of tree condition with the presence of nematodes was not established since heavy populations of *Criconemoides* and *Paratylenchus* were found in trees classed as healthy. A total of 16 trees showing decline symptoms were free from nematodes. Observations at time of sampling and subsequently showed that some of these trees were on poorly-drained sites and root degeneration in these trees could be attributed to unfavorable soil-water relationship. Other possible causes for the condition of these trees are under investigation.

Table 1. Plant-parasitic nematodes in the root zone of selected trees in 10 holly orchards on Vancouver Island.

Orchard No.	1	2	3	4	5	6	7	8	9	10
No. trees	6	9	7	8	7	6	6	7	5	4
Cric.[a]	0	5	7	8	2	0	4	0	0	0
Parat.	0	9	5	1	1	2	2	3	3	0
Prat.	1	2	0	0	0	2	1	7	1	0
Xiph.	0	2	0	1	0	0	0	2	5	0

[a] See footnote a, Table 2.

Table 2. Plant-parasitic nematodes in the root zone of holly trees in relation to tree condition.

	No.	Cric.[a]		Parat.		Prat.		Xiph.		
Tree condition	trees	H.	L.	H.	L.	H.	L.	H.	L.	Free
Poor	36	6	7	4	11	1	6	3	2	15
Pale	4	0	3	0	3	0	1	0	0	0
Bronzed	4	0	0	0	2	0	3	1	2	1
Leaf Drop	2	2	0	0	1	0	0	0	0	0
Healthy	17	2	6	2	3	0	3	0	1	4

[a] Cric. = *Criconemoides annulifer*. max. pop. per 1 lb soil = 1840
Parat. = *Paratylenchus* sp. do. do. = 1700
Prat. = *Pratylenchus penetrans* do. do. = 400
Xiph. = *Xiphinema americanum* do. do. = 200
H. = populations exceeding 100 per 1 lb soil.
L. = populations less than 100 per 1 lb soil.

EXPERIMENTAL FARM, RESEARCH BRANCH,
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[1] Contribution No. 182, Experimental Farm, Research Branch, Canada Department of Agriculture, Saanichton, B. C.

[2] Bosher, J. E. 1954. Root-lesion nematodes associated with root-decline of small fruits and other crops in British Columbia. Can. J. Agr. Sci. 34: 429-431.

REDUCTION OF SUSCEPTIBILITY TO CUCUMBER SCAB BY CUCUMBER MOSAIC VIRUS[1]

H. J. Hopen and D. J. deZeeuw[2]

Summary

Four pickling type cucumber varieties were evaluated for severity of cucumber scab attained when virus-free and virus-infected plants were inoculated with *Cladosporium cucumerinum* Ell. & Arth. Mosaic infection reduced growth of almost all plants and was most severe on the variety Maine-2. Scab infection was less severe on mosaic-infected plants than on the virus-free plants. Protection afforded by the virus was greatest for the scab-susceptible varieties but was evident in several cases also on scab-resistant varieties. Minimal mosaic infection (1 day before scab inoculation) afforded considerably less protection than the longer established infections.

REVIEW OF LITERATURE

Antagonisms between various types of pathogenic and saprophytic organisms have often been observed (15) and protection against a virus disease by the same virus or other viruses has also been reported (5, 10, 11).

Studies on antagonism or symbiosis of fungal and viral pathogens, however, are relatively recent. Müller and Munro (9) reported that the growth of *Phytophthora infestans* (Mont.) d By. was retarded in potato that had been inoculated with potato virus X or Y. Hooker and Fronek (7), on the contrary, showed an increased susceptibility of potato to early blight (*Alternaria solani* (Ell. & G. Martin) L. R. Jones & Grout) when plants were infected with potato virus Y.

Yarwood (16, 17, 18, 19) has studied the association of rust and virus infections. In an early study (16) he reported increased infection by plant viruses of rust infected tissue, possibly due to the increasing number and amount of free amino acids in such tissue. In later work (17, 18) he reported an acquired localized resistance to TMV lesion formation in beans in the vicinity of previously formed TMV lesions, and that rusted bean leaves afforded no protection from several diseases including TMV and the tobacco necrosis virus (18). Wilson (14) showed an increased resistance to rust in TMV-infected bean leaves apparently due to inhibition of uredospore germination. The substances imparting resistance were thought to be water soluble.

Blumer, et al. (2) demonstrated an inhibition of systemic cucumber mosaic in cucumber plants previously infected with mildew. There was also some evidence that prior infection with mosaic was inhibitory to mildew development. Recently Goheen and Schnathorst (6) reported that virus leafroll-affected grapevines had an increased concentration of carbohydrates in the leaves, probably because of phloem necrosis, and increased resistance to powdery mildew. Resistance to mildew increased with an increase in the osmotic value of the tissue.

When cucumber seedlings were screened for resistance to scab and mosaic at Michigan State University, it was necessary to infect with scab first in order to obtain reliable segregation ratios (unpublished data Michigan Cucumber Project, D. J. deZeeuw). Burton (3) and Burton and deZeeuw (4) showed a decrease in susceptibility to scab in cucumber plants having reduced vigor. Burton also demonstrated a rapid depletion of asparagine and glutamine during early stages of scab infection in the more vigorous plants.

MATERIALS AND METHODS

The experiments reported here were carried out in the glasshouse during the spring and autumn of 1961. Four pickling type cucumber varieties (1, 3) with the following disease reactions were used in the study.

Variety	Mosaic	Scab
CS-2M	Resistant	Resistant
CS-2W	Resistant	Susceptible
Maine-2	Susceptible	Resistant
Yorkstate	Resistant	Susceptible

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Several Michigan and Wisconsin isolates of C. cucumerinum were grown on potato-dextrose agar and the spores collected for inoculum approximately 1 week after seeding. Cucumber mosaic virus (Isolate No. 9) from cucumber was maintained on Nicotiana tabacum var. xanthi n. c.; extracted by grinding; and applied to the test plants in .01M phosphate buffer (pH 7.0) plus carborundum with a dental type pressure atomizer (8). Spore suspensions of the scab organism without carborundum were sprayed on test plant leaves with the same type atomizer. After scab inoculation, all plants were held in a 65° F moist chamber for 48 hours. Treatments were replicated four times in most experiments.

In all experiments, except parts of the studies on time intervals, the mosaic inoculations were made in the cotyledonary stage and the scab inoculation at the time of initial expansion of the first true leaf.

Plants were grown in a soil-sand-peat potting mixture and fertilized with water soluble fertilizer to maintain vigorous growth.

RESULTS

Plants inoculated with mosaic 5 days before scab inoculation (Table 1, Exp. 1) were not so severely affected by scab as the plants inoculated with only scab and symptoms were slower in development. The protection given by mosaic infection was observable from the time of the appearance of the initial scab symptoms until senescence. The succeeding four experiments were generally confirmatory.

Experiment 3 compared the infection by scab in mosaic-infected and mosaic-free plants at three growth stages, namely, the time of expansion of the first, second and third true leaves of varieties CS-2M and CS-2W. Mosaic infection again provided some protection from severe scab. Protection was afforded by the mosaic when plants were scab-inoculated in the one-leaf and three-leaf stages. Less protection was observed in the two-leaf set in this test.

The results of experiments 2 and 4 were combined (Table 2) and the effect of time interval between mosaic and scab inoculation was evaluated. Mosaic inoculations were carried out in the cotyledonary stage and in the very early one-leaf stage. Scab inoculations were made while the first leaf was expanding. Plants infected with mosaic showed some local symptoms of scab 3 days after inoculation, while those treated with only the scab showed a rapid development of severe symptoms including terminal necrosis 4 days after inoculation. The longer mosaic establishment (4 days) afforded the greater protection against scab with less necrosis while the minimal time (1 day) appeared to allow little development of the scab protection mechanism (Fig. 1C). This was particularly clear with the variety Yorkstate.

Experiment 5 (Table 3) was conducted during September with plants grown in flats. Larger numbers were used and time intervals of 1, 3, and 5 days between mosaic and scab inoculation incorporated.

Mosaic infection was very severe on the variety Maine-2 throughout and nearly as severe on the other varieties in the 3-day interval group. Plants at this growth stage were displaying the first leaf tip, were very soft, and consequently many were killed by the virus. Symptoms of mosaic were readily apparent in other parts of the test without being as lethal.

Plants of the scab susceptible variety CS-2W were severely diseased by this organism when not previously inoculated with mosaic. Seven (of 60) plants in the 5-day interval group that appeared to have escaped mosaic infection showed susceptibility to scab. In the 3-day interval group the mosaic killing was excessive but the few survivors of the virus had relatively little evidence of scab susceptibility. Where the interval was only 1 day nearly half of the plants (28 of 60) had terminal necrosis from the scab and another 28 had a noticeable resistant reaction. The apparent level of resistance to scab in this case was less than in plants with a longer mosaic establishment but still somewhat higher than in the non-mosaic controls.

Mosaic infection was detrimental to the growth or vigor of the three varieties although the mosaic-resistant varieties were less severely affected (Fig. 1A, 1B). The typical mottle and distortion symptoms were also not so obvious on the resistant varieties. Scab resistant varieties reacted to inoculation with the fungus by the development of restricted lesions or flecks. Although these lesions were generally less noticeable on inherently scab-resistant varieties having mosaic infection than the other mosaic-free counterparts, the differences were not measurable as they were on the scab-susceptible varieties.

The true leaves of scab susceptible plants became epinastic and senescent when infected with scab alone while those previously infected with mosaic generally persisted on the plant in a leathery and wrinkled condition for several days before senescence set in (Fig. 1A, 1B). None of the virus-infected plants were observed to be more severely infected with scab than virus-free plants.

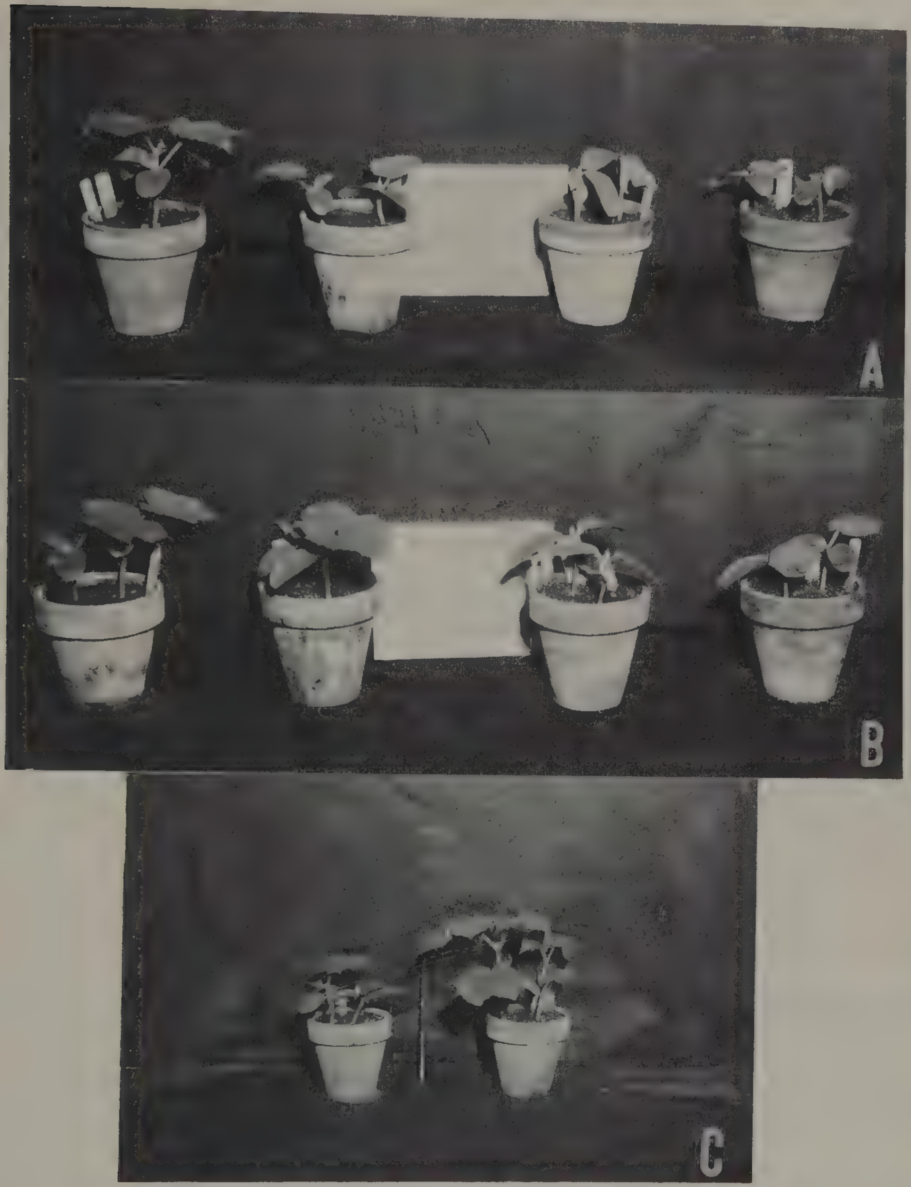

FIGURE 1. Effect of mosaic infection on subsequent development of scab of cucumber seedlings. A -- Variety CS-2W. B --Yorkstate. From left to right, control, mosaic alone, scab alone, mosaic plus scab. C -- Variety CS-2W. Left, mosaic inoculation 1 day before scab. Right, mosaic inoculation 4 days before scab.

DISCUSSION

Infection by CMV appears to make the host substrate less suitable for development of the scab fungus. Minimal mosaic infection afforded considerably less protection than the well established infections.

Müller and Munro (9) also found that virus infection worked against the subsequent development of a fungus disease. They postulated that the potato viruses X or Y affected late blight development through their effects on the host protoplasm. If the virus directly altered only the host's metabolism, it is conceivable that some metabolites essential for the fungus would be eliminated or decreased.

Table 1. Effects of CMV infection of cucumber on subsequent infection by scab.

Inoculation[a]	Variety	Observations[b]
Control	YS	No mosaic. No scab.
	CS-2M	No mosaic. No scab.
	CS-2W	No mosaic. No scab.
	Me-2	No mosaic. No scab.
Mosaic	YS	Mild systemic mosaic. No scab.
	CS-2M	Mild systemic mosaic. No scab.
	CS-2W	Mild systemic mosaic. No scab.
	Me-2	Severe mosaic, stunting. No scab.
Scab	YS	No mosaic. Severe scab of foliage, 4 of 8 plants with terminal necrosis.
	CS-2M	No mosaic. Mild scab (resistant flecks), no t. n.
	CS-2W	No mosaic. Severe scab of foliage, 6 of 8 plants with t. n.
	Me-2	No mosaic. Mild scab (resistant flecks), no t. n.
Mosaic and Scab	YS	Mild systemic mosaic. Mild scab on 5/8, t. n. on 3/8.
	CS-2M	Mild systemic mosaic. Mild scab. No t. n.
	CS-2W	Mild systemic mosaic. Mild scab on 6/8. Severe with t. n. on 2/8.
	Me-2	Severe mosaic, stunting[c]. Mild scab. No t. n.

[a] Inoculated with scab 5 days after mosaic inoculation.
[b] Notes 13 days after mosaic and 8 days after scab inoculation.
[c] Two plants killed by mosaic.

Table 2. Severity of scab infection in relation to stage of mosaic development.

Inoculation	Variety	Observations[a]
Control	YS	No mosaic. No scab.
	CS-2M	No mosaic. No scab.
	CS-2W	No mosaic. No scab.
	Me-2	No mosaic. No scab.
Mosaic	YS	Mild mosaic. No scab.
	CS-2M	Mild mosaic. No scab.
	CS-2W	Mild mosaic. No scab.
	Me-2	Severe mosaic. No scab.
Scab	YS	No mosaic. Severe scab, terminal necrosis on 16/16.
	CS-2M	No mosaic. Mild scab (resistant flecks), no t. n.
	CS-2W	No mosaic. Severe scab, t. n. on 10/16.
	Me-2	No mosaic. Mild scab (resistant flecks), no t. n.
Mosaic 1 day before scab	YS	No mosaic[b]. Severe scab, t. n. on 8/8.
	CS-2M	No mosaic[b]. Mild scab, no t. n.
	CS-2W	No mosaic[b]. Severe scab, t. n. on 3/8.
	Me-2	No mosaic[b]. Mild scab, no t. n.
Mosaic 4 days before scab	YS	Mild mosaic[b]. Moderate scab, t. n. on 5/8.
	CS-2M	Mild mosaic[b]. Mild scab, no t. n.
	CS-2W	Mild mosaic[b]. Moderate scab, t. n. on 3/8.
	Me-2	Severe mosaic. Mild scab, no t. n.

[a] Two experiments combined.
[b] Notes on mosaic symptoms between time of scab inoculation and 4 days later.

Table 3. Effect of 1, 3 or 5 day establishment of mosaic in cucumber on infection with scab.

Inoculation	Variety	No. plants	Observations
Control	CS-2M	60	No mosaic. No scab.
	CS-2W	60	No mosaic. No scab.
	Me-2	57	No mosaic. No scab.
Mosaic 5 days before -- no scab	CS-2M	60	Severe mosaic, stunt on 40. No scab.
	CS-2W	60	Severe mosaic, stunt on 53. No scab.
	Me-2	56	Severe mosaic, stunt on 33. No scab.
Mosaic 3 days before -- no scab	CS-2M	60	Mosaic severe on all, killed 26. No scab.
	CS-2W	60	Mosaic severe on all, killed 10. No scab.
	Me-2	60	Mosaic severe on all, killed 58. No scab.
Mosaic 1 day before -- no scab	CS-2M	60	Moderate mosaic, slight stunt. No scab.
	CS-2W	60	Moderate mosaic, slight stunt. No scab.
	Me-2	60	Severe mosaic but less stunt than above. No scab.
Scab only	CS-2M	60	No mosaic. Few resistant scab flecks.
	CS-2W	60	No mosaic. All severe scab, terminal necrosis (t. n.) on 25.
	Me-2	60	No mosaic. All mild scab, few resistant flecks.
Mosaic 5 days before scab	CS-2M	60	Severe mosaic, stunt on 50. Few resistant scab flecks.
	CS-2W	60	Severe mosaic & stunt on 53 that had few scab flecks. 7 mosaic escapes had t. n. by scab.
	Me-2	60	Severe mosaic & stunt on 29; 31 with mild mosaic or escaped. No scab symptoms.
Mosaic 3 days before scab	CS-2M	60	Mosaic killed 28, 30 severe mosaic and stunt. No scab evident.
	CS-2W	60	Mosaic killed 27, 33 severe mosaic and stunt. Few resistant flecks, no t. n. from scab.
	Me-2	60	Mosaic killed 57. No scab symptoms.
Mosaic 1 day before scab	CS-2M	60	Moderate mosaic, slight stunt on all. A few resistant scab flecks.
	CS-2W	60	Moderate mosaic, slight stunt. Scab t. n. on 28; resistant scab flecks on 28.
	Me-2	60	Severe mosaic & stunt on 60. No scab symptoms.

The present experiments were not designed to ascertain the mechanism responsible for the protection against growth of Cladosporium cucumerinum in the host. It is probable, however, that nutrient materials required for scab growth were depleted or converted by development and multiplication of the virus. Burton and deZeeuw (4) showed that scab susceptible cucumbers lost asparagine and glutamine rapidly during the infection process, presumably in conjunction with a carbohydrate accumulation in the leaves, and resistant cucumbers did not. The resistant plants had no vascular disruption that should have led to large carbohydrate accumulation and had not lost the two characteristic amides. It seems possible that rapid parasitism by scab is dependent on a carbohydrate accumulation after the initial invasion. In the case of virus leafroll of grape (6), a vascular disruption is apparently responsible for carbohydrate accumulation in the leaves. Powdery mildew, in contrast to some other fungus pathogens, is inhibited by the increased osmotic concentration resulting from this buildup. Cucumber mosaic is not a virus that typically affects phloem and probably does not lead to scab resistance through an increased osmotic concentration. Cladosporium cucumerinum is a well adapted saprophyte and plants affected with mosaic are undoubtedly deficient in some of the nutrients required by the fungus. The expression of susceptibility by rapid necrosis or extensive lesioning is thus restrained.

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MICHIGAN AGRICULTURAL EXPERIMENT STATION, EAST LANSING

BACTERIAL LEAF STRIPE OF SWEET CORN[1]

C. Wehlburg[2]

Abstract

A bacterial leaf disease of sweet corn causing yellow or tan elongated irregular blotches and stripes especially on the lower leaves occurs naturally on peat soils of the Everglades. From the symptoms on the host plant and the physiological behavior of the pathogen it was concluded that the cause of the disease is Pseudomonas andropogonis (E. F. Sm.) Stapp. The disease is not considered serious on commercial varieties of sweet corn.

INTRODUCTION

A bacterial leaf disease of sweet corn was observed in late spring and again in the fall of 1960 in the Everglades. Elliott and Smith (3, 4) described a similar bacterial disease of sorghum and broomcorn called bacterial stripe, and named the causal agent Bacterium andropogonis E. F. Sm. Recently Ullstrup (6) reported the disease occurring naturally on field corn and proved that it was caused by the same bacterium: Pseudomonas andropogonis (E. F. Sm.) Stapp.

In this paper the symptoms of the disease on sweet corn and the laboratory tests that characterize the pathogen are described.

SYMPTOMS

The symptoms as they are found in the field are elongated blotches that may coalesce to form irregular areas of affected tissue.

In the beginning the spots are olive green, water-soaked, and somewhat translucent. As the tissue dies the color of the blotches progressively turns yellow, orange, and finally bright tan (Fig. 1). The spots appear almost exclusively on the lower leaves and the disease can be recognized from a distance by the typical bright yellow and brown color of the lower layer of leaves. Microscopic examination of a small strip of diseased tissue in a drop of water showed bacteria oozing out in great numbers.

THE PATHOGEN

Several isolates from sweet corn were grown in pure culture on beef-peptone, lima bean and potato-dextrose agar. Growth was usually slow. Lima bean agar was found to be the best medium for propagation. The organism is a short rod, 1.8 to 2.4μ x 0.5 to 0.8μ, motile by one polar flagellum, Gram negative, not acid fast, and does not produce fluorescin on the proteose peptone No. 3 agar recommended by King, Ward and Raney (5). Colonies on beef-peptone agar are round, raised, and glistening, with entire margins, white by reflected light, and a delicate tan with lighter margin by transmitted light. On nutrient broth growth is slow, producing a slight turbidity, a thin pellicle, and a light precipitate after 48 hours. It does not liquefy gelatin or decompose corn seed. Milk turned slightly alkaline after 3 days, without clearing or coagulating. Nitrates were not reduced, nor was there any production of indole or H_2S. Starch hydrolysis was moderate; ammonia was produced. No acid or gas was formed from sucrose or lactose. These data agree closely with descriptions in Bergey's Manual (1) and Elliott (2), indicating that the pathogen is Pseudomonas andropogonis (E. F. Sm.) Stapp.

ARTIFICIAL INOCULATION

Greenhouse grown corn plants, 2 to 3 weeks old, were inoculated in the following ways: 1) The unopened leaf in the whorl was clipped off and a drop of bacterial suspension in water was placed on the stump. 2) The stem was punctured with a needle through a drop of the suspension. 3) A drop of suspension was placed in the whorl. 4) The plants were sprayed with the suspension.

The plants were kept in a humid atmosphere for 48 hours after inoculation. The first symptoms usually showed after 4 days as water-soaked, olive green elongated spots. During the next few days they grew larger and turned a dull brown. In the greenhouse the infected areas never showed the orange-yellow and bright tan color that is so typical of the disease in the field on older leaves. Method 1 and 2 were considered too strong as many young plants broke at the site of perforation with method 1 and sometimes, with either method, the emerging leaves were brown and rotted. In some cases the young leaves that came out after the affected ones were completely colorless. They regained their color of normal green within 2 weeks.

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The methods of inoculating without wounding the tissue gave desirable results, especially if the plants were kept in a moist chamber for 24 hours prior to inoculation.

The disease did not spread from infected leaves to new leaves that developed later.

Several varieties of sweet corn were used in the greenhouse studies; Gold Rush and Iobelle 104 and popcorn A-KP-3881 had the clearest symptoms.

The pathogen was repeatedly re-isolated from inoculated leaves that showed the symptoms of the disease.

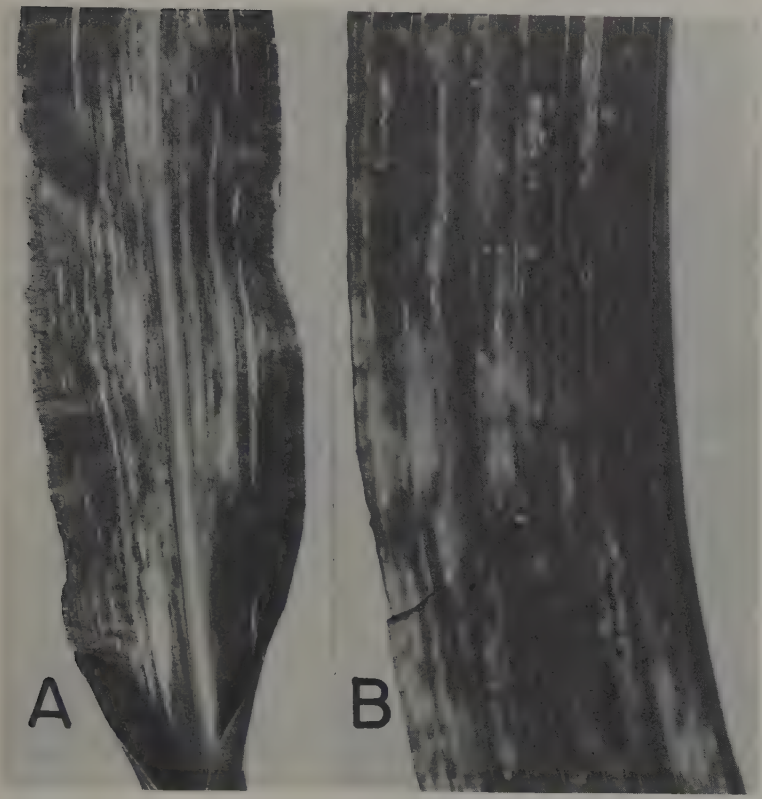

FIGURE 1. A -- Leaf of sweet corn showing symptoms of bacterial stripe. B -- The same symptoms enlarged about 1 1/2 X.

DISCUSSION

Bacterial leaf stripe occurs on all varieties of sweet corn in the Everglades under humid conditions. Nevertheless, Elliott and Smith (3) reported that they failed to extend the host range to corn. Neither the chlorotic striping and bleaching of the leaf blade nor the distortion of the upper internodes described by Ullstrup (6) were observed on sweet corn in the field. Bleaching of the young leaves occurred only in the greenhouse after very severe wound inoculation. Although severely affected leaves may die prematurely, the disease is limited to the lower portion of the plants and does not seem to be of much importance for sweet corn in the Everglades.

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EVERGLADES EXPERIMENT STATION, BELLE GLADE, FLORIDA

DIFFERENTIAL SENSITIVITY TO SODIUM ORTHOPHENYLPHENATE BY BIPHENYL-SENSITIVE AND BIPHENYL-RESISTANT STRAINS OF PENICILLIUM DIGITATUM

Paul R. Harding, Jr.[1]

Abstract

Critical concentrations of sodium orthophenylphenate in nutrient agar were selective for biphenyl-resistant strains of citrus green mold (*Penicillium digitatum* Sacc.), that is, were inhibitory to growth of biphenyl-sensitive strains but not of biphenyl-resistant strains. In tests run with lemons, residues of sodium orthophenylphenate left in the uninjured rind by typical prestorage tank treatment were selective for the biphenyl-resistant strain in lemons injured and inoculated with the two strains separately and mixed after treatment. Tests run with nutrient agar showed that biphenyl-resistant strains can develop from biphenyl-sensitive strains submitted to sublethal concentrations of sodium orthophenylphenate. The extent to which such mutations may occur on treated lemons in storage has not been determined.

The high percentages of biphenyl-resistant green mold spores found in the green mold spore populations of lemon packing houses where sodium orthophenylphenate has been used continuously in prestorage tank treatments have evidently resulted from the selectivity of the chemical for biphenyl-resistant strains, regardless of whether such strains originated by mutation induced by the chemical or by spontaneous mutation.

INTRODUCTION

Sodium orthophenylphenate has been used in washing tank solutions for the past several years by a number of California lemon packing houses for control of Penicillium decay during storage. Van der Plank and Rattray (6) showed that solutions of this chemical will effectively disinfect injuries in harvested citrus fruits against *P. digitatum*, the causal organism of green mold decay. Hopkins and Loucks (3) and Long and Roberts (4) developed improved solutions for effective application of the chemical without injury to the fruit.

Lemons are usually received from the orchards in stages of dark green to light green and require a period of storage for development of proper color and texture. The lemons are washed before going into storage. Some packing houses have continuously used sodium orthophenylphenate in their washing tank solutions as a preventative to mold decay during storage. Following storage, decays are culled out -- which are always appreciable in number despite the prestorage use of sodium orthophenylphenate -- and those lemons appearing sound are packed in fiberboard paper cartons containing two sheets of paper impregnated with biphenyl. Biphenyl is a vapor phase fungistat used to protect citrus fruits against decay and dusty sporulation caused by blue and green Penicillium molds. Lemon packing houses that have used sodium orthophenylphenate continuously in prestorage washing tanks have been realizing little or no control of green mold (*P. digitatum*) decay in lemons shipped with biphenyl treatment.

The kinds of biphenyl resistance developed by citrus blue (*P. italicum*) and green (*P. digitatum*) molds have been described by Harding (1). Since biphenyl and orthophenylphenol are related in that both contain in their chemical structures the same double-ring configuration, the writer conceived the possibility that lemon packing house strains of *P. digitatum* resistant to biphenyl (2) might be resistant also to critical concentrations of orthophenylphenol as applied in the form of its water-soluble salt sodium orthophenylphenate. Sodium orthophenylphenate as used in hot water washing and treating tanks in some lemon packing houses effectively sterilizes rind injuries acquired before treatment. This treatment, however, leaves a lesser residue in the uninjured rind which may be selective for biphenyl-resistant strains in injuries acquired after treatment. Whether this chemical could be responsible for the buildup of the high percentages of biphenyl-resistance in the green mold populations of lemon packing houses is the object of this investigation.

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MATERIALS, METHODS, AND RESULTS

Selectivity of Sodium Orthophenylphenate in Agar for the Biphenyl-Resistant Strains: For the *in vitro* part of this study, agar (Difco potato-dextrose agar supplemented by 0.2% yeast extract and 0.2% neopeptone) plates were prepared containing different concentrations of sodium orthophenylphenate. Growth rates in millimeters per 24 hours for the linear phase were determined at the different concentrations for five monosporially-derived biphenyl-resistant and five monosporially-derived biphenyl-sensitive strains of *P. digitatum*. Inoculation was performed by transferring a 4-mm loopful of concentrated (about 10 million spores per cc) suspension to the agar plate. All plates were held at 70° F for the run of the experiment. Owing to the buffering action of neopeptone, variation in the pH of the agar was little (6.25 to 6.55) as the concentration of sodium orthophenylphenate was increased from zero to 160 ppm. The results of this part of the experiment are shown in Table 1, where growth rates are given as averages of four replications.

Table 1. Growth rates in millimeters per 24 hours for biphenyl-resistant and biphenyl-sensitive strains of *Penicillium digitatum* submitted to different concentrations of sodium orthophenylphenate in nutrient agar.

Strain	Biphenyl nature	Concentrations of sodium orthophenylphenate (in ppm) 0	5	10	20	40	80	160
866	resistant	11.5	11.5	10.0	7.3	3.9	2.1	0.0
1141	do.	11.0	11.0	9.8	6.8	3.8	1.9	0.0
1115	do.	12.0	12.0	10.5	7.0	3.9	1.8	0.0
1156	do.	11.0	11.3	10.0	7.0	3.9	1.7	0.0
1254	do.	10.6	10.5	9.9	7.2	4.0	2.0	0.0
1077	sensitive	9.7	5.0	3.0	1.5	0.0	0.0	0.0
1138	do.	9.0	4.5	3.0	1.3	0.0	0.0	0.0
1102	do.	9.5	5.3	3.2	1.5	0.0	0.0	0.0
1142	do.	10.8	5.5	3.5	1.7	0.0	0.0	0.0
1143	do.	10.0	5.3	3.5	1.5	0.0	0.0	0.0

In Table 1, growth cessation lies between 20 and 40 ppm for biphenyl-sensitive strains, and between 80 and 160 ppm for biphenyl-resistant strains. At other concentrations of sodium orthophenylphenate, growth in the resistant strains was much less retarded than in the sensitive strains. Sporulation in the sensitive strains was little or did not develop at 20 ppm, but did develop abundantly at the lower concentrations. Sporulation in the resistant strains was little or did not develop at 80 ppm, but did develop abundantly at the lower concentrations.

Mutants Cross Resistant to Biphenyl: During the course of the study of growth rates, sectorial mutations in the sensitive strains submitted to sodium orthophenylphenate in agar were fairly common. For example, at 20 ppm (where the sensitive strains were slow-growing and showed little or no sporulation) resistant strains arose from sensitive strains as faster-growing sporulating sectors on some of the colonies. Similar resistant sectors with growth rates faster than the sensitive parent colonies arose at 10 ppm of sodium orthophenylphenate in the agar. Subculturing and testing revealed that some of the variants obtained in this manner were not true mutants, that is, did not result from permanent genetic change, but lost their resistance to the chemical after subculturing on nutrient agar lacking the chemical. Nevertheless, a number of true mutants with permanent resistance to the chemical were obtained. Several of the mutants were tested for cross resistance to biphenyl vapor. This was accomplished by inoculating lemons with the different strains, and holding the lemons at 58° F for 1 week in nonvented cartons containing the conventional 2-sheet biphenyl treatment[2]. Inoculation consisted of making a scratch 1.0 cm long and 1.0 mm deep on the rind and painting the scratch with a soft brush dipped in a suspension having a concentration of about 10 million spores per cc. Two cartons of 165 lemons each were used per strain, one carton biphenyl-treated, the other nontreated. All fruits were randomized before inoculation. Decay potentials in percentage were determined on the basis of the number of fruits decayed. The results of this work are shown in Table 2.

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[2] Commercial biphenyl treatment consists of two sheets of No. 48 Crown-Kraft paper impregnated with a mixture containing biphenyl. One sheet is placed in the bottom and the other in the top of the carton. Each sheet is 10 9/16 x 16 1/4 inches and contains 2.35 g of biphenyl in a mixture of biphenyl 50%, paraffin 25%, mineral oil 24%, and lemon oil 1%.

Table 2. Degrees of cross resistance to biphenyl by strains of *Penicillium digitatum* mutantly resistant to sodium orthophenylphenate, as shown in nonvented cartons of inoculated lemons stored for 1 week at 58° F.

Mutant strain	Parent strain	Treatment	% decay potential of mutant strain	% decay potential of parent strain
1216	1138	biphenyl	93.94	10.30
		none	75.76	100.00
1218	1102	biphenyl	49.70	11.52
		none	100.00	100.00
1221	1142	biphenyl	60.61	9.09
		none	100.00	100.00
1241	1077	biphenyl	100.00	8.48
		none	100.00	100.00
1245	1143	biphenyl	100.00	9.70
		none	100.00	100.00

In Table 2, mutant 1216 was stimulated by biphenyl. In addition to the larger number of decayed lemons resulting from biphenyl treatment in the carton, a more advanced stage of decay was reached. Mutants 1218 and 1221 are partially resistant to biphenyl, and mutants 1241 and 1245 are completely resistant. The difference between partial resistance and complete resistance was reflected by more progress of decay as well as by the larger numbers of fruits decayed in the latter case. Biphenyl suppressed sporulation in the biphenyl-sensitive parent strains, but not in the mutants.

Whether mutations occurring in the presence of sublethal concentrations of sodium orthophenylphenate are induced by the chemical or are spontaneous has not been determined. Resistant strains could develop through selection of either induced or spontaneous mutations.

No definite determination has yet been made of the extent to which biphenyl-resistant mutants may develop from biphenyl-sensitive strains on lemons in packing houses where sodium orthophenylphenate is used in prestorage washing tank treatments. This matter is under investigation.

Selectivity of Sodium Orthophenylphenate Residue in the Lemon Rind for Biphenyl-Resistant Strains: The use of sodium orthophenylphenate in prestorage washing and treating tanks has been a continuous practice in some California lemon packing houses. Tests using a standard method (7) showed that the rind of light green lemons may take up 10 to 50 ppm of this chemical from a typical tank treatment followed by thorough rinsing. To investigate the possibility of a selective effect of this residue for biphenyl-resistant strains of *P. digitatum*, uninjured lemons were put through a typical tank treatment consisting of immersion for 3.5 minutes in an 0.8% solution of sodium orthophenylphenate maintained at 115° F and having a pH of 11.8 (adjusted by adding sodium hydroxide). The high pH is necessary to avoid chemical injury to the rind. This treatment was followed by rinsing in tap water at 70°. Eight replications of 50 lemons each were treated and rinsed in this manner. For comparison, 8 replications of 50 lemons each were put through another typical tank treatment consisting of immersion for 3.5 minutes in a 3.0% solution of sodium carbonate (pH 11.5) maintained at 115°. This treatment was followed by rinsing in tap water at 70°. All fruits were randomized before treatment. All samples were injured and inoculated after the treatments and rinsing, by making a scratch 1.0 cm long and 0.5 mm deep on the rind of each lemon and then applying spores to the injury with a soft brush. In this manner, of the eight replications treated with sodium orthophenylphenate, four replications were inoculated with biphenyl-resistant green mold (strain #1141) and four replications were inoculated with biphenyl-sensitive green mold (strain #1101). The lemons treated with sodium carbonate were inoculated in the same arrangement. Each replication was placed in a storage box, stored at 58° for 1 week, and the percentage of decay in each then determined on the basis of the number of fruits decayed. The results of this experiment are shown in Table 3.

In Table 3, the preinoculation treatment with sodium orthophenylphenate left a residue in the rind which gave a much lower average percentage of decay with the biphenyl-sensitive strain (21.0%) than with the biphenyl-resistant strain (71.0%). By the Lord (5) range test, this difference is significant to the 99.9% confidence level. By the same method of statistical analysis, a significant difference does not exist between the average percentages of decay in the controls (the two strains with the preinoculation treatment of sodium carbonate). These results show that a residue of sodium orthophenylphenate as left in the uninjured rind by a typical tank treatment could be selective for biphenyl-resistant strains in lemons acquiring injuries after such treatment while being processed for storage. The percentage of biphenyl-resistant green mold

Table 3. Differential tolerance for rind residue of sodium orthophenylphenate by biphenyl-resistant and biphenyl-sensitive strains of Penicillium digitatum on lemons.

Mold strain	Preinoculation treatment	No. fruits	No. decayed	% decay	Average % decay
resistant	sodium orthophenylphenate	50	29	58.00	
do.	do.	50	40	80.00	
do.	do.	50	35	70.00	
do.	do.	50	38	76.00	71.00
sensitive	do.	50	7	14.00	
do.	do.	50	15	30.00	
do.	do.	50	8	16.00	
do.	do.	50	12	24.00	21.00
resistant	sodium carbonate	50	40	80.00	
do.	do.	50	30	60.00	
do.	do.	50	36	72.00	
do.	do.	50	42	84.00	74.00
sensitive	do.	50	32	64.00	
do.	do.	50	29	58.00	
do.	do.	50	37	74.00	
do.	do.	50	32	64.00	65.00

Table 4. Results of replica plating to show how selective effect of sodium orthophenylphenate residue will increase percentage of biphenyl-resistant green mold spores on lemons decaying during storage.

Inoculum	Preinoculation treatment	Run no.	Approx. No. spores tested	% of spores biphenyl-resistant
99% sens. and 1% res.	3% sodium carbonate	1	20,000	1.11
		2	20,000	0.84
100% sens.	3% sodium carbonate	1	20,000	0.00
		2	20,000	0.00
99% sens. and 1% res.	0.5% sodium orthophenylphenate	1	20,000	2.74
		2	20,000	2.10
100% sens.	0.5% sodium orthophenylphenate	1	20,000	0.00
		2	20,000	0.00
99% sens. and 1% res.	0.8% sodium orthophenylphenate	1	20,000	5.36
		2	20,000	4.73
100% sens.	0.8% sodium orthophenylphenate	1	20,000	0.00
		2	20,000	0.00

could therefore buildup in storage, particularly on recycling of this pattern.

When weaker fruit is used, the selective effect of sodium orthophenylphenate in the lemon rind may not be reflected significantly by the numbers of decayed fruits when samples treated with sodium orthophenylphenate are compared with samples treated with sodium carbonate. Nevertheless, the selective effect is easily demonstrable by using mixed inoculum and replica plating. In this technique, each of three samples of 100 lemons each was given a different treatment as follows: 1) Immersion for 3.5 minutes in 3.0% solution of sodium carbonate (pH 11.5) maintained at 115° F. 2) Immersion for 2 minutes in 0.5% solution of sodium orthophenylphenate (pH 11.8) maintained at 90°. 3) Immersion for 3.5 minutes in 0.8% solution of sodium orthophenylphenate (pH 11.8) maintained at 115°. Each treatment was followed by rinsing in tap water at 70°. All samples were injured and inoculated after the treatments and rinsing, by making a scratch 1.0 cm long and 0.5 mm deep on the rind of each lemon and then painting the scratch with a soft brush dipped in a concentrated suspension of spores (about 10 million per cc). For these three samples, mixed inoculum was used, containing biphenyl-sensitive and biphenyl-resistant green mold spores in a proportion of 99% sensitive to 1% resistant. For comparison and check on possible mutations, three more samples, each of which had been given a different one of the three treatments, were inoculated in the same manner but using inoculum comprised 100% of biphenyl-sensitive green mold spores. All fruits were randomized before treatment.

Each sample was stored in a paper sack at 58° until decay was advanced enough to show abundant sporulation (2 weeks). The spores were collected from each sample and the percentage of biphenyl-resistant spores determined. Each sample was assayed according to the following procedure. The spores were suspended in water, and each suspension diluted to 2500 spores per cc so that when 0.4 cc was pipetted to an agar plate, about 1000 spores were introduced. The inoculum was spread over the agar by tilting and rotation. Twenty replications with biphenyl in the lids (15 grams per lid caked by melting and cooling) and one check plate without biphenyl, were used per treatment. These agar plates were held at 70° for 4 days and the number of biphenyl-resistant colonies then counted (only resistant spores form colonies). This entire experiment was run twice. The results are shown in Table 4.

In Table 4, sodium carbonate is nonselective, that is, approximately the same percentage of biphenyl-resistant spores was found in the decayed lemons as was used in the inoculum. On the other hand, sodium orthophenylphenate is definitely selective in that it consistently caused considerable increase in the percentage of biphenyl-resistant green mold spores. Obviously, recycling as would occur in a lemon packing house where lemons are washed, stored, culled of decays, and then packed, could increase the resistant strain to a high percentage. This explains why high percentages (20 to 60%) of the green mold spore populations are biphenyl-resistant in lemon packing houses where sodium orthophenylphenate has been used continuously in prestorage tank treatments, and why such packing houses have obtained little or no control of green mold decay and sporulation in cartons of lemons shipped with the conventional biphenyl treatment. By contrast, lemon packing houses that have never used sodium orthophenylphenate in prestorage treatments have low percentages (zero to less than 1.0%) of biphenyl-resistant strains in the green mold spore populations, and consequently realize good decay control with biphenyl in shipments.

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OIL-SPRAY TRIALS ON CERCOSPORA DISEASES OF TOBACCO, PEANUT, ZINNIA, AND GRAMALOTE GRASS

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Abstract

Low-volume oil applications that controlled Cercospora musae on banana leaves did not control Cercospora diseases on tobacco and zinnia, and gave only slight control on peanut and gramalote grass. A continued search is advisable for other plant diseases that may be controlled by spray oil.

Oil sprays for fungus disease control are a relatively new development in plant pathology. They are used by banana growers in the form of low-volume mist applications to control the Sigatoka leaf-spot disease. The pathogen's imperfect stage is Cercospora musae Zimm. Oils are also known to control at least two more Cercospora diseases: greasy spot of citrus and leaf spot of tung (2, 3). It would be of interest to determine whether oil sprays can control still other Cercospora diseases, especially on crops that are adapted to low-volume applications. The present paper describes the results of oil-spray trials on four different hosts attacked by Cercospora species.

The pathogens and hosts used in these trials were as follows: Cercospora nicotianae Ell. & Ev. on tobacco (Nicotiana tabacum var. olor[4]), Cercospora zinniae Ell. & Martin on zinnia[5] (Zinnia sp.), Cercospora personata (Burk. & Curt.) Ell. & Ev. on peanut (Arachis hypogaea var NC4[6]), Cercospora fusimaculans Atk. on gramalote grass (Panicum maximum var. gramalote). Each host species was arranged in plots approximately 20 x 30 feet with the individual plants set 3 feet apart. Cercospora musae on Dwarf Cavendish bananas was used as the check. During the experiment, the plants were cultivated and received sufficient water and fertilizer to ensure good growth.

Each plant of the test species to be treated was covered with a rectangular plastic box 4 feet tall and 2 feet square at the base and top. The bottom of the box was open. An artist's airbrush[7] was used to spray the oil through a small hole at the top of the box. The oil mist was confined within the box; no drift occurred onto adjacent plants. The oil[8] was one of many known to give good control of C. musae on banana leaves. Treatments were: no oil spray, 0.45 ml per plant, and 1.8 ml per plant. The spray rates were equivalent to 1.3 and 5.1 gallons per acre, respectively[9]. Each treated plant was sprayed at weekly intervals. There were 15 replicate plants per treatment arranged in a randomized block design. At the time of the first oil application, all the plant parts to be treated were young and had very few or no disease symptoms.

As a comparative control the youngest unfurled leaf on five banana plants was sprayed at weekly intervals using the low deposit rate (1.3 gallons/acre) and a spray technique described previously (1). Other similar banana leaves were chosen as unsprayed checks.

All observations on disease incidence were made 10 weeks later, except for the zinnias which were observed at 7 weeks because the plants were beginning to die due to the disease. For the tobacco and zinnia, all of the Cercospora spots per plant were counted and the average number of spots per leaf was determined. One shoot from each clump of gramalote grass and five shoots from each peanut plant were chosen at random, the spots were counted and the average number per leaf calculated. Two trials were carried out and the results are summarized in Table 1.

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[1] Plant Pathologist.

[2] Agronomist.

[3] Formerly Assistant Officer in Charge, Federal Experiment Station, now Chief, Tobacco and Sugar Crops Research Branch, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

[4] Obtained from the Agricultural Experiment Station of the University of Puerto Rico, Río Piedras, Puerto Rico.

[5] Royal Purple, from the W. Atlee Burpee Co., Philadelphia, Pennsylvania.

[6] Obtained from Prof. W. C. Gregory, North Carolina State College of Agriculture, Raleigh, North Carolina.

[7] Obtained from the Paasche Airbrush Co.; nozzle no. 3 was used.

[8] A paraffinic oil, visc. 88.5 SUS at 100° F, u. r. 100%, supplied by the Esso Research and Engineering Co., Linden, New Jersey.

[9] ml/4 sq. ft. were converted to gallons/acre.

Table 1. Effect of spray oil[a] on the control of five Cercospora leaf-spot diseases[b].

Trial no.	Peanuts (gallons/acre) 0.0	Peanuts 1.3	Peanuts 5.1	Gramalote grass (gallons/acre) 0.0	Gramalote grass 1.3	Gramalote grass 5.1	Tobacco (gallons/acre) 0.0	Tobacco 1.3	Tobacco 5.1	Zinnia (gallons/acre) 0.0	Zinnia 1.3	Zinnia 5.1	Bananas (gallons/acre) 0.0	Bananas 1.3
1	11	13	11	12	10	10	14	11	13	18	17	17	310	0
2	5	3	3	15	5	5	62	64	63	11	11	9	440	0

Average number of Cercospora spots per leaf[c]

[a] Paraffinic oil, visc. 88.5 SUS at 100° F, u.r. 100% supplied by Esso Research and Engineering Co., Linden, New Jersey.

[b] Six to 10 applications at weekly intervals beginning with new healthy plant growth. Two spray rates were used.

[c] Duncan's multiple range test; values underscored with a line are not significantly different at the 5% level. No differences occurred at the 1% level of significance.

There were no significant differences in disease incidence between the oil treatments and checks for zinnia and tobacco in either of the trials. The oil-treated gramalote and peanut plants, on the other hand, had significantly fewer spots than the checks (at the 5% level of significance) in one of the trials. The treated banana leaves had no spots, while each check-leaf had over 300 spots.

No visible phytotoxic symptoms due to the oil were present on the test species. Since in one trial the treated gramalote and peanut plants had significantly less disease than the nontreated plants, an additional check for phytotoxicity was made in these cases. On the one hand, the height of the gramalote shoots were measured; while on the other hand, the fresh and dry weights of the aboveground part of the peanut plants were determined. No differences were found between treated and unsprayed plants, thereby suggesting that phytotoxic effects were not present. In bananas, however, some phytotoxicity was apparent in the form of flecks on the leaves that appeared about 6 weeks after the initial spray.

The results of these trials show that tobacco and zinnia did not respond to the particular oil tested at the rates used. There was a suggestion of control in some of the trials with peanut and gramalote grass, but in no sense can this be compared with the reproducible excellent control of C. musae on banana provided by the low-volume oil applications. The limited disease control exerted on the former hosts suggests that different oil types and rates of application should receive further consideration.

No symptoms of phytotoxicity were apparent in peanuts, gramalote, tobacco, or zinnia but some were found on banana leaves sprayed with oil for the control of C. musae. A low threshold of phytotoxicity may be necessary to control Cercospora diseases by oil.

In conclusion, under the conditions of these tests oil did not adequately control the Cercospora diseases of tobacco, peanut, zinnia, or gramalote grass, even though applied at rates above those needed to control C. musae on bananas. However, a continued study of oil for the control of plant diseases is well deserved because of its low cost, convenience of application, and its known effectiveness against Cercospora attacking bananas, citrus, and tung trees.

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TREE KILLING BY FOMES ANNOSUS IN A GENETICS ARBORETUM

Robert V. Bega[1]

Abstract

Recent large-scale killing of pines in the Institute of Forest Genetics arboretum at Placerville, California has been found to be caused by Fomes annosus. Particularly significant was the diversity of pine species attacked: 24 species, 3 varieties, and 1 artificial hybrid, including representatives of the genus Pinus, subgenus Diploxylon, from the entire northern hemisphere. There was no evidence of aerial spread of the fungus or of degrees of resistance in the pine species. The aggressiveness demonstrated by the fungus in this arboretum shows that it can be a major pest in future forest plantations in California.

The fungus Fomes annosus (Fr.) Cke., causal agent of one of the most important diseases of conifers in Europe, has been reported as killing trees in plantations of southern and eastern United States and eastern Canada (1, 2, 4, 7). In California it is common and often destructive in natural forests. As a tree killing pathogen it is most common on pines. In white fir (Abies concolor), California red fir (A. magnifica), and in other conifers the fungus causes a root and butt rot but seldom directly kills the tree.

A rapidly expanding outbreak of the disease has killed large numbers of trees within a relatively short period of time in the Eddy Arboretum of the Institute of Forest Genetics, Placerville, California. The disease in this arboretum has provided an unparalleled opportunity for comparing, in a relatively uniform habitat, the susceptibility to the fungus of a wide range of pine species of the subgenus Diploxylon.

The Institute of Forest Genetics is situated on the west slope of the north-central Sierra Nevada at an elevation of 2,700 feet. The soil is a deep, Aiken clay-loam. The arboretum, founded in 1925, contains what is probably the most complete collection of pines in the world. The collection (before the F. annosus outbreak) included more than 70 species, 35 varieties, and 90 different hybrids. The area is divided into a main arboretum in which the various species are located and contiguous areas on which provenience, progeny, and hybrid tests are underway. The Eddy Arboretum therefore serves as a tree breeding laboratory and as a place where students, botanists, visiting scientists, and laymen can observe and study the species and hybrids (3). In the part of the main arboretum where F. annosus is at present most damaging, the trees are from 32 to 34 years old; height and diameter range from 25 feet tall and 4.5 inches dbh (diameter at breast height) for the slow growing species to 64 feet tall and 15 inches dbh for the faster growing species. Spacing between trees is 15 feet, which is much greater than in those areas of eastern and southeastern United States where the disease is active.

EXTENT OF INFECTION

Tree killing by F. annosus was first noticed in the main arboretum in 1951[2]. At that time two Douglas-firs (Pseudotsuga menziesii) and four Italian stone pines (Pinus pinea) were involved. In 1953 these trees were removed, and the 3,500-square foot infection area was fumigated with carbon disulfide.

From February 1960 to September 1961, I examined dead and dying trees in the same arboretum (Figs. 1 and 2) and found that F. annosus had again become active in isolated pockets. Each was associated with stumps resulting from routine thinning operations and from periodic removal of individual trees killed by adverse climatic conditions or by, at the time, unknown cause. Several stumps were sound enough for examination and were found to be heavily infected with F. annosus. The thinning and sanitation operations had been done just before and after the 1951 F. annosus discovery.

Of particular significance in this infection was the diversity of pine species attacked. Table 1 lists these species and their natural distribution. They included representatives from the entire northern hemisphere. Figure 2 outlines the pockets of infection with the exception of the one hybrid which was found in another portion of the main arboretum not shown on this map. Up to

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[1] Plant Pathologist, Pacific Southwest Forest and Range Experiment Station, Berkeley, California.

[2] Personal communication from P. C. Lightle and Willis W. Wagener.

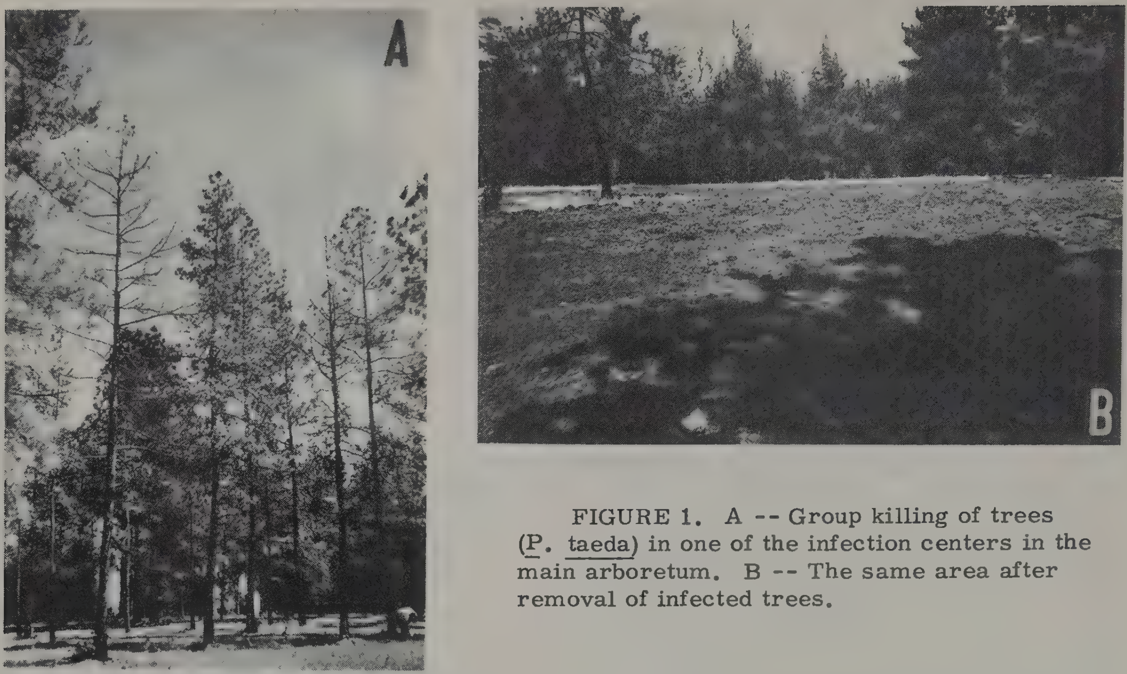

FIGURE 1. A -- Group killing of trees (P. taeda) in one of the infection centers in the main arboretum. B -- The same area after removal of infected trees.

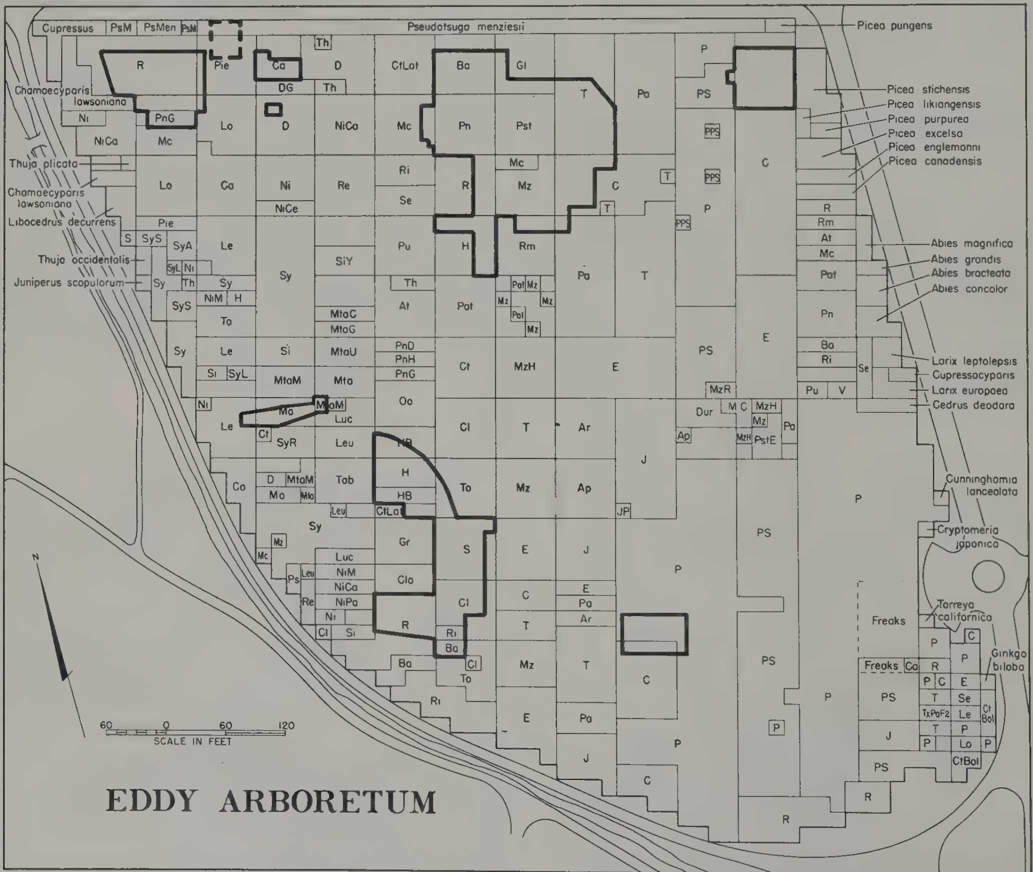
Cupressus
PsM
PsMen
Pseudotsuga menziesii
Picea pungens
Chamaecyparis lawsoniana
Thuja plicata
Chamaecyparis lawsoniana
Libocedrus decurrens
Thuja occidentalis
Juniperus scopulorum
Picea stichensis
Picea likiangensis
Picea purpurea
Picea excelsa
Picea englemanni
Picea canadensis
Abies magnifica
Abies grandis
Abies bracteata
Abies concolor
Larix leptolepsis
Cupressocyparis
Larix europaea
Cedrus deodara
Cunninghamia lanceolata
Cryptomeria japonica
Torreya californica
Ginkgo biloba
Freaks
N
60 0 60 120
SCALE IN FEET
EDDY ARBORETUM

FIGURE 2. Diagram of that part of the main arboretum in which F. annosus is active. Infection centers are outlined in heavy black. Area delineated by dashes indicates 1951 infection center. Species symbols are found in Table 1. (Original map taken from (3))

Table 1. Pine species infected with *F. annosus* in the Eddy Arboretum and their natural distribution. (Compiled from (3) and (5))

Scientific and common names	IFG symbol[a]	Natural distribution
1. Pinus banksiana Jack pine	Ba	Across southern Canada and northern United States from Maine to Minnesota.
2. P. brutia (=P. halepensis-brutia) Erectcone Aleppo pine	Br(=HB)	Northern Mediterranean region.
3. P. canariensis Canary Island pine	Ca	Canary Islands, 5,000 to 7,000 feet.
4. P. caribaea Caribbean pine	C	Western West Indies, northern Central America, and southeastern Mexico.
5. P. coulteri	Cl	Coastal mountains of central and southern California. Northern Baja California.
6. P. densiflora Japanese red, Akamatsu	D	Japan, South Korea, and west-central China.
7. P. glabra Spruce pine	Gl	Southeastern United States.
8. P. halepensis Aleppo pine	H	Mediterranean region.
9. P. leiophylla Smoothleaf pine, Chihuahua	Le	Mexico.
10. P. massoniana Masson: Chinese red	Ma	Southeastern and south-central China.
11. P. montana var. mughus Mugo pine	MtaM	Europe: eastern Alps and mountains of Balkan States.
12. P. montezumae Montezuma pine	Mz	Mexico and northern Central America.
13. P. muricata Bishop pine	Mc	Discontinuous along coast of northern and central California. Also Pt. San Quentin in Baja California and the Islands of Cedros and Guadelupe.
14. P. palustris Longleaf pine	Pa	Southeastern United States.
15. P. pinaster Maritime pine, cluster pine	Pn	Northern Mediterranean region and Atlas Mountains of North Africa.
16. P. pinaster var. gigantea	PnG	Mediterranean region.
17. P. pinea Italian stone pine	Pie	Northern Mediterranean region.
18. P. ponderosa Ponderosa pine	P	Widely distributed in western North America: British Columbia to northern Mexico.
19. P. ponderosa var. scopulorum. Rocky Mountain ponderosa pine	PS	United States: eastern Nevada to east of the Continental Divide.
20. P. pseudostrobus False Weymouth pine	Pst	Subtropical southern Mexico and northern Central America.
21. P. radiata Monterey pine	R	Coast of central California, Santa Cruz Islands, Santa Rosa Islands. Guadelupe Island off coast of Baja California.
22. P. remorata (considered by some to be a variety of P. muricata)	Re	Confined to Santa Rosa and Santa Cruz Islands of California.
23. P. rigida Pitch pine	Ri	New Brunswick and southern Ontario south to Georgia and Tennessee.
24. P. roxburghii (= P. longifolia) Chir pine	Lo	Himalaya Mountains from Bhutan to Afghanistan.
25. P. sabiniana Digger pine	S	Lower slopes of foothills around Central Valley of California.
26. P. taeda Loblolly pine	T	Southeastern United States.
27. P. torreyana Torrey pine	To	Limited to a small coastal area north of San Diego, California, and on Santa Rosa Island.
28. P. xattenuradiata Knobcone x Monterey pines	AtR	A hybrid developed at the Institute of Forest Genetics, Placerville, California.

[a] Institute of Forest Genetics symbols.

September 1961, 24 species, 3 varieties, and 1 artificial hybrid of pines were involved in the infection area.

DISCUSSION

The large number and holoarctic representation of pine species in this infection center is significant. None of the species attacked showed any evidence of resistance. Unless the fungus is controlled, it could eventually attack and kill all species in the main arboretum. (Control measures now being employed will be reported in a subsequent paper.) It is further assumed that these species are being attacked by the same clone of the fungus. This point is now under investigation.

All evidence indicates that the present infected area was the result of a single initial infection established in one of two P. pinea trees (Fig. 2) some time before 1951. There is no way of ascertaining now whether the original infection was from a chance spore, from inoculum carried in by a bark beetle or borer or on a piece of equipment such as a chain saw. The indication of spread from a single source is supported, however, by the absence of the fungus from other parts of the Institute grounds where the opportunity for stump infection by airborne sources prevails.

Because of the rarity of fruit-body (conk) production by the causal fungus under California conditions and because of the limited pattern of spread in the arboretum, infection was probably not a result of airborne spores. Occasional fruit bodies have been found in California (6), but these were under a layer of duff and litter completely hidden from sight; consequently, the chance of aerial dispersal of basidiospores is greatly minimized. The relative scarcity of fruit bodies in California may be due to the low summer humidities in the areas where the fungus is prevalent. Of the large number of infected trees and stumps examined in the Institute of Forest Genetics Arboretum, only one small (20 mm diameter) fruit body was found, and it was beneath a 15-cm layer of litter. Willis W. Wagener of this office has offered the hypothesis that fungus spread in the arboretum may have been accomplished by means of the chain saw used in thinning and sanitation operations. Experiments are now being conducted to test this hypothesis. Once an infection center was started, further spread was shown to be accomplished by root contact between diseased and healthy trees.

Although several of the susceptible pine species reported in this paper are undoubtedly new host records for the fungus, no attempt has been made to prove this point.

This report supports the prediction (6) that as virgin timber diminishes with cutting in western United States, and as plantation forestry becomes more prevalent, the importance of F. annosus will increase rather than decrease.

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GLOMERELLA CINGULATA AND MYROTHECIUM SPP. IN SEEDS OF SMALL GRAINS[1]

W. F. Crosier

Abstract

The storage of Glomerella cingulata, Myrothecium roridum and M. verrucaria in the seeds of barley, oats and wheat is reported for the first time. Supplemental inoculum consisting of species of Cephalosporium, Helminthosporium, Phoma or Nigrospora did not prevent establishment of G. cingulata and M. verrucaria. H. monoceras is also reported as a successful inoculant of Avon wheat heads.

INTRODUCTION

Glomerella cingulata (Stonem.) Spauld. & Schrenk has been reported as seed borne in cocoa, coffee, Hibiscus spp., lupin, sorghum, sweet pea and tea (7). The fungus has not been isolated from seeds of any of these plant species in New York State. Prior to 1960 neither G. cingulata nor any species of Colletotrichum had been observed on seeds of small grains.

Myrothecium spp. were stated (2, 7) to infect apples, bells of Ireland, cowpea, onion, potato, squash, tomato and turnip. A leaf spot of gardenia and a crown rot of hollyhock, pansy and snapdragon have been attributed to M. roridum Tode ex Fr. (4, 7). It was listed by Noble, et al. (6) as being seed transmitted in tomato. M. verrucaria (Alb. & Schw.) Ditm. ex Fr. was not named in the annotated list of seed-borne diseases.

Pure cultures of Glomerella cingulata and of Myrothecium spp. are regularly used at Cornell University (3) and elsewhere (1, 5) in the microbiologic assaying of suscept treated seeds.

MATERIALS AND METHODS

The fungi under study were cultured on malt-dextrose or Sabouraud's agar contained in either Petri dishes or test tubes. The fungal growths and agar were blended with equal volumes of Pyrax and pressed into the maturing spikelets of barley, oats and wheat. The inoculated plants were held at 23°C in a mist chamber for at least 50 hours.

The ripened heads were hand threshed and the seeds stored temporarily at 3°C. Either 100 or 150 seeds were surface-sterilized in 2% sodium hypochlorite and plated on potato-malt-dextrose agar. The plates were held at 20°C under alternating fluorescent lights until the fungal colonies could be identified.

When the inoculum was only one fungus, 15 seeds were plated in each Petri dish. If the inoculum consisted of two fungi, only 10 seeds were placed in a plate.

RESULTS

The first use of Glomerella cingulata as an inoculant of small grains arose from its inadvertent mixing with about 10 times its mass of Helminthosporium sorokinianum Sacc. ex Sorokin. When 100 ripened seeds of the host, Russell wheat, were plated on sterile agar, three colonies of G. cingulata occurred among 98 of H. sorokinianum.

During 1960 and 1961 Glomerella cingulata was stored in the seeds of several varieties of barley, oats and wheat. The fungus-seed associations resulted from the inoculation of immature grains with G. cingulata alone or in a mixture with another fungus. As shown by a few data in Table 1, G. cingulata was established within seeds in competition with the rapidly growing fungi, Alternaria tenuis Nees ex Wallr., Nigrospora oryzae (Berk. & Br.) Petch and N. sphaerica (Sacc.) Mason. A. tenuis and G. cingulata were frequently present in the same seed and produced separate colonies on nutrient agar (Fig. 1).

An inoculum of Glomerella cingulata and Myrothecium verrucaria established these species in seeds of Erie barley (Tables 1 and 2). The latter fungus alone was stored in seeds of four varieties of barley, oats and wheat. It also became seed borne under conditions not satisfactory for the establishment of graminicolous pathogens (Fig. 2).

When a mixture of Myrothecium verrucaria and Helminthosporium monoceras Drechsl. was pressed into heads of Avon wheat, both fungi became resident in the mature seeds. These two species together and occasionally either one alone were the only fungi isolated from ripened seeds

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[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1286.

Table 1. Storage of *Glomerella cingulata* in seeds of small grains.

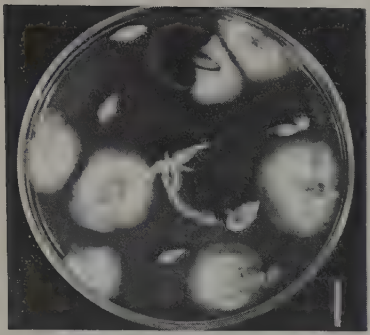
Supplemental inoculum, species	Variety and kind of host plant	Seeds (%) carrying internally				
		Glomerella cingulata	Other inoculant	Alternaria tenuis	Nigrospora spp.	Other fungi
None	Garry oats	0	--	0	1	9
	Oneida oats	14	--	0	3	0
	Russell wheat	2	--	0	9	7
Cephalosporium gramineum	Red Bobs wheat	13	1	8	0	8
Helminthosporium bromi	Erie barley	39	0	3	0	1
	Hudson barley	18	0	18	6	0
	Garry oats	14	0	0	6	6
Helminthosporium cyclops	Oneida oats	0	4	0	18	2
Helminthosporium gramineum	Garry oats	4	0	14	10	0
	Red Bobs wheat	2	0	14	0	12
Helminthosporium sorokinianum	Russell wheat	3	98	0	0	0
Helminthosporium siccans	Erie barley	17	0	38	3	2
Myrothecium verrucaria	Erie barley	17	3	38	3	2
Phoma insidiosa	Erie barley	64	4	0	6	3
	Oneida oats	2	0	2	2	0
	Avon wheat	31	0	0	0	0
	Selkirk wheat	95	0	0	0	0

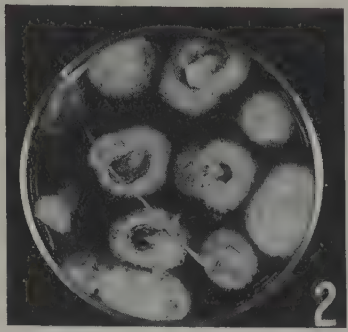
Table 2. Storage of *Myrothecium* spp. in seeds of small grains.

Supplemental inoculum placed on heads	Variety and kind of host plant	Seeds (%) carrying internally				
		Myrothecium species	Other inoculant	Alternaria tenuis	Nigrospora spp.	Other fungi
Myrothecium verrucaria						
None	Erie barley	54	--	3	0	1
	Garry oats	1	--	2	13	0
	Avon wheat	36	--	0	0	10
	Genesee wheat	65	--	0	21	0
Cephalosporium gramineum	Erie barley	47	0	0	0	0
	Oneida oats	2	0	2	16	0
Glomerella cingulata	Erie barley	3	18	45	2	3
Helminthosporium bromi	Garry oats	2	0	0	6	0
Helminthosporium monoceras	Avon wheat	68	12	0	0	0
Helminthosporium siccans	Erie barley	3	0	45	2	3
Myrothecium roridum						
None	Erie barley	74	--	0	0	12
	Selkirk wheat	2	--	36	0	2

of Avon wheat. Usually, however, *Alternaria tenuis* and/or *Nigrospora* spp. were also present internally in seeds harvested from heads of barley, oats or wheat which had been inoculated with *M. verrucaria* alone or with another fungus.

Myrothecium roridum isolated from pansy was a successful inoculant of Erie barley and of Selkirk wheat. It produced spores in typical sporodochia on the seeds as well as on the agar substrate (Fig. 3).





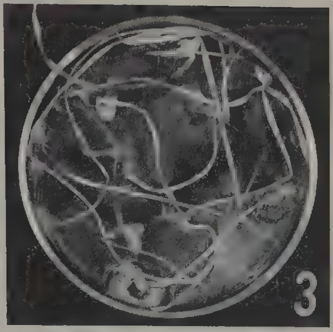


FIGURE 1. *Glomerella cingulata* (Stonem.) Spauld. & Schrenk and *Alternaria tenuis* Nees ex Wallr. are occasionally co-residents of seeds of Erie barley.

FIGURE 2. *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr. developing from seeds of Erie barley.

FIGURE 3. *Myrothecium roridum* Tode ex Fr. and *Alternaria tenuis* Nees ex Wallr. developing from seeds of Selkirk wheat.

DISCUSSION

Since *Glomerella cingulata* and *Myrothecium* spp. are not highly specific pathogens, it is not surprising that they will become associated with seeds of small grains. The routine testing of about 12,000 lots of feed and seed grains for internal fungi had failed to reveal these fungi prior to 1960. Neither fungus has been observed on other tissues of barley, oats, rye or wheat. It is conjectured, however, that species of *Glomerella* and *Myrothecium* are present in small grains and in these vehicles are being carried to all land areas.

It is not generally recognized that *Nigrospora oryzae* and *N. sphaerica* are seed borne in small grains. Noble, et al. (6) name these fungi as residents of only corn and rice seed, although they are occasionally found in wheat seed grown in the northeastern States. Apparently environmental conditions favorable for the establishment of *Nigrospora* spp. in seeds also permit *Glomerella cingulata* and *Myrothecium* spp. to become seed borne. The fact that *Nigrospora* spp. have occurred in wheat seed produced commercially in the Northeast whereas neither *G. cingulata* nor *Myrothecium* spp. has been observed indicates that inocula of the latter species are wanting, not that they lack aggressiveness.

Since *Glomerella cingulata* and *Myrothecium verrucaria* are commonly used in the microbiologic assaying of fungicide treated seeds, it is suggested that small grains are convenient storage facilities for the fungi. When protected by grains, the fungi may be safely transported and preserved for at least 2 years. It is known that *G. cingulata*, *M. verrucaria* and *Helminthosporium sorokinianum* may be stored in the same seed and later recovered as pure cultures.

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LEAFHOPPER VECTORS OF VIRUS DISEASES IN MICHIGAN

J. R. Hoffman[1] and O. Taboada[2]

In an attempt to determine the leafhopper vectors of plant virus diseases in the central Michigan area, collections of leafhoppers were taken at about 8-day intervals from May until after killing frosts in the late fall. These collections were carried out over an 8-year period.

The collections produced 93 species of leafhoppers from selected collecting sites. These sites included *Rubus* sp., *Crataegus* sp., *Prunus serotina*, sour cherry and grasses. Of the 93 species of leafhoppers determined from the collections, 10 are recognized as vectors of plant virus diseases.

Not all of the diseases the leafhopper vectors may carry occur in Michigan. Examples of such diseases are wheat striate mosaic virus and sugarcane chlorotic streak virus. The leafhopper vectors are responsible for spreading some of the more economically important plant virus diseases such as aster yellows and peach yellows virus. The knowledge of the presence of potential vectors of plant virus diseases is valuable from the standpoint of being forewarned.

The following is a list of the known plant virus disease carrying leafhoppers found in the collections.

Leafhopper	Vector for virus disease	Occurs in Michigan
Aceratagallia sanguinolenta (Prov.)	Potato Yellow Dwarf Virus (3)	Yes
Agallia constricta V. D.	Potato Yellow Dwarf Virus (3)	Yes
	Clover Wound Tumor Virus (3)	No
Agallia quadripunctata (Prov.)	Potato Yellow Dwarf Virus (3)	Yes
	Clover Wound Tumor Virus (3)	No
Colladonus clitellarious (Say)	X-Disease of Peach and Cherry (1)	Yes
Draeculacephala portola Ball	Sugarcane Chlorotic Streak (2)	No
Endria inimica (Say)	Wheat Striate Mosaic Virus (10)	No
Macropsis trimaculata (Fitch)	Peach Yellows Virus (6, 7)	Yes
	Peach Little-Peach Virus (7) (probably)	Yes
Macrosteles fascifrons (Stål)	Aster Yellows (5)	Yes
Neokolla hieroglyphica (Say)	Alfalfa Dwarf Disease Virus (4)	No
	(Pierce's Disease of Grape) (4)	No
Scaphytopius acutus (Say)	Peach Western X-Disease Virus (1, 8)	No
	Peach X-Disease Virus (Suggested vector) (1, 8)	Yes
	Alfalfa Witches' Broom Virus (9)	No

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RELATION BETWEEN THE EFFECT OF BIPHENYL ON GERM TUBE DEVELOPMENT AND CONTROL OF CITRUS GREEN MOLD AMONG ISOLATES OF PENICILLIUM DIGITATUM

Ruben Duran[1]

Abstract

The detrimental effects of biphenyl vapors on germ tube development were established for several hundred biphenyl-sensitive isolates of Penicillium digitatum Sacc.

Biphenyl control of green mold in lemons was predictable from in vitro tests, depending on the reaction of different isolates to biphenyl. Spores of sensitive isolates distended abnormally or produced hypertrophied germ tubes, whereas spores of non-sensitive isolates germinated normally.

INTRODUCTION

While demonstrating differential sensitivity to biphenyl among isolates of Penicillium digitatum Sacc., Duran and Norman[2] reported sensitive isolates could be detected by the hypertrophic effect of the chemical on germ tube development. This phenomenon, if consistent, could be useful in several ways: 1) the sensitivity of pure cultures could be easily determined in the time normally required for spore germination (usually less than 24 hours at room temperature), 2) semi-quantitative evaluations of sensitive and non-sensitive populations of P. digitatum in citrus packing houses could be made, and 3) since growth rate is affected by substrate, temperature, and strain variability, the undesirable aspect of using differences in radial growth for judging sensitivity could be eliminated.

To determine whether this phenomenon could be used to separate consistently sensitive from non-sensitive isolates, several hundred stock cultures were tested. The results of these tests and other findings are reported.

MATERIALS AND METHODS

The test material, 296 isolates collected over a 2-year period, was obtained by exposing Petri plates containing potato-sucrose agar in packing houses in Los Angeles, Orange, San Bernardino, San Diego, Santa Barbara, and Ventura counties.

Spores collected in the packing houses were incubated 24 hours at room temperature and subsequently placed in a biphenyl chamber, described by Duran and Norman[2], for 72 hours. Mycelial transfers were made, from colonies that developed in the chamber, to potato-dextrose agar slants. Inasmuch as other penicillia (P. chrysogenum, P. frequentans, P. italicum, P. notatum) and other contaminants frequently developed in the slants, single-spore isolations of P. digitatum were subsequently made (by micro-manipulation) of all cultures even though some appeared to be uncontaminated. Every test isolate was thus represented by a clone derived from one spore.

After sporulation, four spores from each test isolate were micro-manipulated onto four agar blocks. Each series of blocks representing a test isolate was placed on a sterile glass coverslip and suspended over biphenyl crystals in a Van Tiegham cell and kept at room temperature. Each isolate was thus represented by four treated spores. After recording the effects of biphenyl on spore germination, one spore or sporeling was transferred to an agar slant to provide a stock culture for subsequent use as inoculum. The remaining spores were discarded.

Spores that produced rapidly elongating germ tubes in biphenyl were readily established when transferred to agar slants and kept at room temperature; under similar conditions spores that distended or developed misshapen germ tubes usually failed to grow when transferred to slants. To obtain inocula of these isolates, other spores were germinated in the absence of biphenyl.

Spores that in germinating became distended or developed malformed germ tubes under biphenyl were tentatively considered to represent sensitive isolates; similarly, spores able to

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[2] Duran, Ruben and Shirley M. Norman. 1961. Differential sensitivity to biphenyl among strains of Penicillium digitatum Sacc. Plant Disease Reptr. 45: 475-480.

Table 1. Relation between control of citrus green mold in artificially inoculated lemons and the effects of biphenyl on germ tube development of 296 isolates *in vitro*.

Isolates tested[a]	Spores tested	Spores germinated[b]		Isolates controlled in treated lemons
		normal	abnormal	
255 (non-sensitive)	1020	749	none	none
41 (sensitive)	164	none	129	all

[a] Inoculum of sensitive isolates produced in the absence of biphenyl inasmuch as spores originally treated with biphenyl failed to produce cultures.
[b] Spores were considered to have germinated if they produced a germ tube or swelled to the degree described in Figure 2.

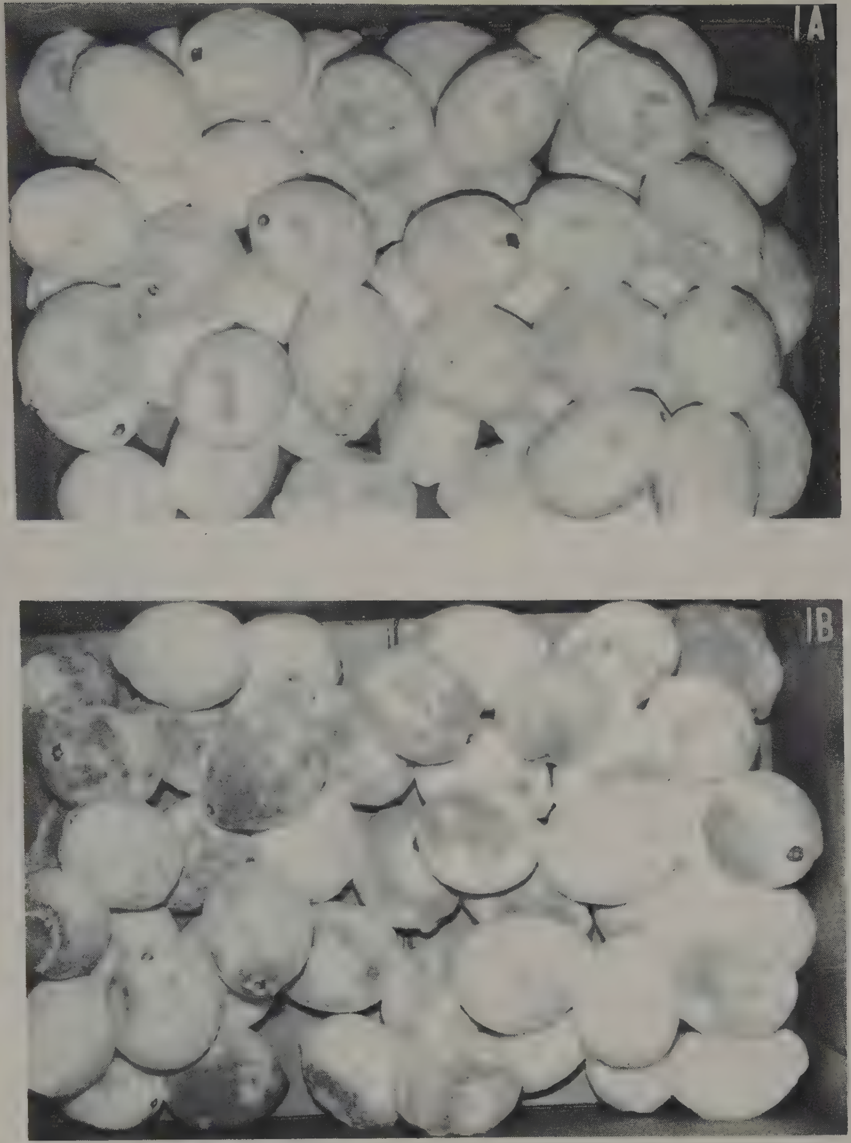
Table 2. Variation in decay, surface mycelium, and sporulation in lemons artificially inoculated with 296 isolates of *Penicillium digitatum* held 1 week at 68° F in nonvented cartons with two biphenyl-impregnated kraft sheets.

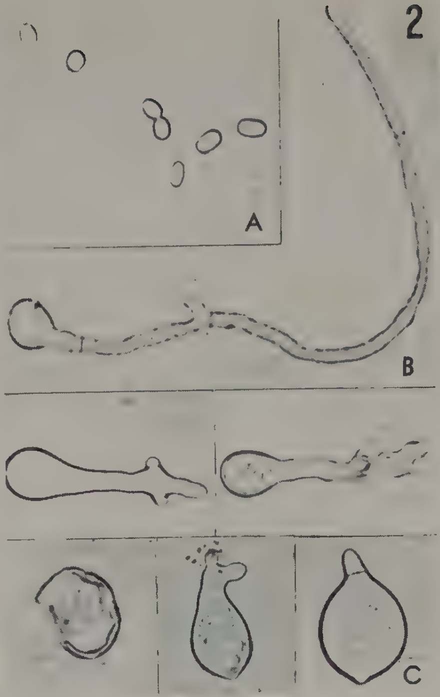
Decays per isolate in lots of 10 inoculated lemons	Number of isolates	Fruits with water-soaked lesions only (approximate surface)	Fruits with aerial mycelium but no spores (approximate surface)	Fruits covered with spore-masses (approximate surface and density)
Sensitive				
0	17	0	0	0
1	14	6 (2-at wound) (1-1/3 fruit) (1-1/4 fruit) (2-1/2 fruit)	7 (1/2 fruit)	1 (1/4 fruit) very sparse
2	4	3 (1/2 fruit)	2 (1-1/4 fruit) (1-1/2 fruit)	3 (1-1/2 fruit) very sparse (2-1/4 fruit) very sparse
3	4	7 (3-at wound) (1-1/4 fruit) (1-1/3 fruit) (2-1/2 fruit)	5 (3-1/2 fruit) (1-3/4 fruit) (1-all fruit)	0
5	1	0	2 (1/2 fruit)	3 (restricted to wound)
6	1	5 (1/3 fruit)	1 (1/2 fruit)	0
Non-sensitive				
9	1	0	9 (1-1/4 fruit) (6-1/3 fruit) (2-1/2 fruit)	0
10	4	0	40 enveloped	0
10	250	0	0	2500 (2/3-3/4) extremely heavy

develop elongated, rapidly branching germ tubes were considered non-sensitive isolates.

To confirm or negate this hypothesis each test isolate was used to inoculate 10 surface-sterilized lemons. The point of a sharp teasing needle laden with spores from pure cultures of each isolate was used to inoculate the fruit.

The inoculated fruits were positioned on egg-case flats to prevent contact between fruits and placed in non-vented fiberboard cartons. A sheet of kraft paper impregnated with biphenyl was placed in the bottom of each carton and another on the fruit. One carton was used to accommodate test fruits of two isolates.





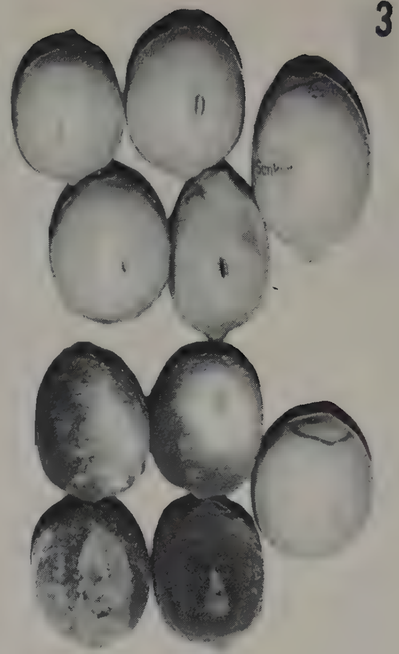


FIGURE 1. Decayed lemons inoculated with a mycelial strain of *Penicillium digitatum* held 1 week at 68° F. A -- Treated with biphenyl. B -- Same strain untreated. Note the general scarcity of sporulation even in the untreated lemons, due more to low sporulative ability of the strain than to the effects of biphenyl (Compare with Fig. 3).

FIGURE 2. Spores of a sensitive isolate. A -- Spores prior to germination. B -- Normal sporeling developed in the absence of biphenyl. C -- Germ tubes showing varying degrees of hypertrophy and distended spores typical of biphenyl effects on spore germination. (Spores of non-sensitive isolates germinate normally under biphenyl and are indistinguishable from the normal development shown in B).

FIGURE 3. General differences in decay of lemons inoculated with biphenyl-sensitive isolates (upper) and non-sensitive isolates (lower) of *Penicillium digitatum*. Note contrasts in symptomatology, that is, restricted necrotic lesions produced by sensitive isolates versus the advanced decay and copious spore masses produced by non-sensitive isolates. Control fruits (wounded but uninoculated) marked at stem ends. (Note the absence of lesions at wound sites).

Inoculated fruit was stored at 68° F and 90% relative humidity and examined after 1 week. The number of decayed fruits, lesion size, amount of surface mycelium, and the approximate surface of inoculated fruits covered by spore-masses were criteria used for judging biphenyl sensitivity among the isolates tested.

RESULTS AND DISCUSSION

The relation between the effects of biphenyl on germ tube development and subsequent disease control in 41 isolates that proved sensitive and 255 that proved non-sensitive is summarized in Table 1.

Early in the tests it became evident that spores of some isolates germinate normally in the presence of biphenyl and produce filamentous, rapidly branching germ tubes. Furthermore, isolates able to develop normal germ tubes in the presence of biphenyl were characteristically virulent to lemons despite treatment with biphenyl. Among these were four mycelial-type isolates (Fig. 1).

By way of contrast, isolates whose spores did not germinate normally in biphenyl were remarkably well controlled when lemons inoculated with these isolates were treated with biphenyl (Compare, Fig. 2 and 3).

The degree of disease control, from the standpoint of decay reduction, varied among sensitive strains. Within the 1-week holding period 17 isolates failed to cause decay, whereas, others caused various degrees of decay (Table 2). Generally, however, the differences between the two types of isolates were apparent (Fig. 3).

The majority of isolates that developed in the biphenyl chamber proved non-sensitive to biphenyl in treated lemons. These results suggest that the conditions in the chamber suppressed the development of sensitive strains but had no effect on development of non-sensitive strains. The results clearly show that single spores of sensitive isolates, although capable of germinating in biphenyl, can not develop a thallus under these conditions. Yet a few sensitive isolates were obtained from the biphenyl chamber. To reconcile these differences it is necessary to postulate that viable material of sensitive isolates was carried along with mycelia of non-sensitive isolates during the mycelial transfers. During the single-sporing process, test isolates of either type could have been established, depending upon the spore selected. Aside from this "inconsistency," *in vitro* tests make biphenyl sensitivity among isolates of *P. digitatum* predictable and relate relative sensitivity to subsequent disease control in treated lemons.

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CULTURE OF APICAL MERISTEMS OF FRAGARIA VESCA STRAWBERRY PLANTS AS A METHOD OF EXCLUDING LATENT A VIRUS[1]

R. O. Belkengren and P. W. Miller[2]

Summary

Stem tips and runner tips of Fragaria vesca infected with latent A virus were removed from two groups of parent plants; one group had received a prior heat treatment, the other had not. Tips were removed so as to include growing points having as little tissue as possible. The tips were then cultured under aseptic conditions on nutrient media until the plants were mature enough to be grown in soil in the greenhouse. They were then indexed by leaf-petiole grafts to detect the presence of latent A virus. Seven of 13 plants so indexed gave no indication of virus at the first indexing. Six plants of the seven were from parent plants that had received a prior heat treatment. The other plant was from a non-heat treated source. Four of these plants have been indexed 10 times in 2 1/2 years and have remained free from latent A virus as determined by the excised leaf graft indexing technique.

INTRODUCTION

The possibility of ridding plants of viruses by culturing excised terminal meristems with and without prior heat treatment of the infected plants has been the subject of a number of investigations.

Bawden (2) states that "the physiology of meristematic cells seems to be such that it either precludes virus infection or, at most, supports virus multiplication poorly. Many viruses do not invade apical meristems, or if they do, they fail to survive there. Virus-free plants might be regenerated from plants infected with such viruses by excising their apical meristems and culturing them in vitro."

Quak (11) excised tips from heat-treated carnations to obtain virus-free plants. Stone (12) used this method to get rid of the ring-spot virus in carnations. Grant (5) used it to get rid of tristeza virus in budwood of a number of varieties of orange. Simple excision without heat treatment has been used successfully by a number of investigators, namely Morel and Martin (9) on dahlia, Kassanis (6) on potato, and Limosset and Cornuet (7) on plants infected with tobacco mosaic. The method appears to have rather wide application, and has been recommended by Bawden (2) for rapidly elongating tissues. Mellor and Fitzpatrick (8) have been able to free cultivated strawberry plants of the latent A virus by propagation of axillary crown buds excised from heat-treated plants even though they found this virus to be resistant to heat inactivation.

It is evident from this review of the literature that excision of apical meristems from infected plants and rooting them independently is one way of excluding certain viruses. However, one of the difficulties encountered by almost all of these investigators is the high mortality rate occurring during the process of rooting.

The investigations reported here represent an attempt to decrease the mortality rate and to get rid of certain strawberry viruses that have been difficult to exclude by other methods.

MATERIALS AND METHODS

Fragaria vesca (East Malling clone) plants infected with the latent A virus were used exclusively in these experiments.

About 50% of the infected mother plants were first heat-treated at 38° C for as long as the plants would endure without dying (generally from 3 to 5 days). After 4 to 6 days, stem and young runner tips were excised. In some cases, the runners were scarcely more than buds in the axil of the leaf. Under a binocular dissecting microscope, as many of the leaves and leaf primordia as possible were removed from the tip of the stem. The tissue was promptly surface sterilized in a solution of sodium hypochlorite (available chlorine 0.02%) for 10 minutes and drained on sterile filter paper in Petri dishes. With a bit of razor blade held in a suitable holder (hemostat forceps are ideal) approximately 0.5 to 1.0 mm of tissue was removed from the end of the stem. The excised fragment was implanted on solid culture medium to such a depth that only the tip protruded from the agar. The medium consisted of White's (13) inorganic salt solution to which 2% sucrose, 10% coconut milk, and 0.7% agar were added. Immediately before sterilization 1/2 mg of indoleacetic acid was added for each liter of medium. Culturing was done either in 125-ml Erlenmeyer flasks or in 2-ounce large mouth prescription bottles (French squares).

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[2] Associate Professor of Botany, Oregon State University, and Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, respectively.

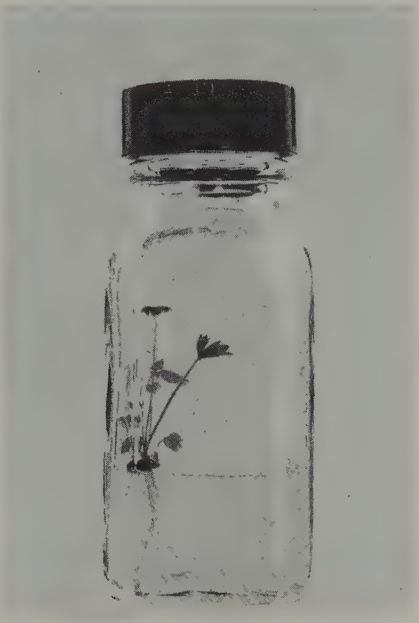

FIGURE 1. An alpine hybrid vesca strawberry plant reared in sterile culture media from an excised runner tip taken from a vesca plant infected with the crinkle virus. Photographed by H. H. Millsap 95 days after implant was made.

Usually the tip cultures were left in darkness for about a week and then placed in continuous light of about 200 ft-c intensity under fluorescent lamps. Daylight will serve just as well, but direct sunlight must be avoided because of heating effects. Likewise, incandescent lamps are not suitable for plants in a closed container. When the plants reached a height of about 1 cm they were transferred to a medium containing only mineral salts and agar, and grown until they were large enough to transplant to sterilized sand and finally to sterilized soil (Fig. 1).

Later, when the plants from the excised parts were large enough they were indexed for the presence of the latent A virus by grafting excised leaves therefrom to alpine hybrid vesca plants infected with either the veinbanding or mottle virus (2, 3). Since the latent A virus is sometimes hard to detect, repeated indexing was done over a 2 1/2-year period to be sure of virus freedom of the plants.

RESULTS AND DISCUSSION

About 30% of all the buds cultured grew into mature plants. In some cases, such a small piece of stem was removed that the meristem was missed entirely or failed to grow. A small percentage of the cultures were lost from contaminants. High temperatures at the time the plants were transferred to the sand and fungus infection caused loss of some plants. Of the original lot of 46 meristems cultured, 13 survived to the point that they could be tested for the presence of the latent A virus.

At the time of the first indexing, 7 of the 13 plants indexed appeared to be free from the latent A virus. Of these, 6 were from mother plants that had been given a heat treatment. Only one plant was from the group that had received no previous heat treatment. Following the first indexing, 3 of the 6 plants from heat-treated sources were lost during greenhouse sanitation. Repeated indexings of the remaining plants over a 2 1/2-year period failed to produce the visual symptoms associated with the presence of the latent A virus when leaves from infected plants were grafted to vesca plants infected with the veinbanding and/or mottle virus.

This study indicates that plants free from the latent A virus may be obtained by culturing excised young runner- and stem-tip meristems; the efficiency of the method appears to be increased if the mother plants are heat-treated just prior to removal of the meristems.

Success in propagating apical meristems is apparently roughly proportional to size of the tissues involved. The chance of obtaining virus-free material is apparently inversely proportioned to size. Heat-treating the mother plants probably allows larger meristems to be taken which are not virus-infected.

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FUNGI INVOLVED IN THE SEEDLING DISEASE COMPLEX OF COTTON IN THE YAZOO-MISSISSIPPI DELTA

C. D. Ranney[1]

Summary

The cotton seedling disease complex is a major disease of cotton in the Yazoo-Mississippi Delta. During the past 6 years this disease complex caused annual losses of $2,958,000.

Surveys in 1959, 1960 and 1961 at four locations indicated that species of four genera of fungi account for 92.5% of the disease complex: *Rhizoctonia solani* 63.6%, *Pythium* spp. 7.1%, *Fusarium* spp. 12.5%, and *Glomerella gossypii* 9.4%. Other fungi, including species of *Alternaria*, *Aspergillus*, *Penicillium* and *Thielaviopsis*, are responsible for 7.5% of the disease complex.

The seedling disease complex is a major cotton disease in the Yazoo-Mississippi Delta. Evaluations of data presented by the Committee on Disease Losses of the Cotton Disease Council (2,3,4,5,6,7) show that an average of 2.04% of the potential Mississippi crop was lost to seedling diseases annually in 1955-1960, representing an accumulated loss of 176,550 bales for the 6-year period. In Mississippi approximately two-thirds of the total cotton production comes from the Yazoo-Mississippi Delta, resulting in an average yearly loss of 19,720 bales in the 15-county Delta area. At a nominal value of $150 per bale this would result in an annual cash loss of $2,958,000.

Research on cotton seedling disease control has shown that a knowledge of the specific organisms involved and the frequency of their occurrence is essential as control measures under one disease regime may not be effective against another. A similar survey in Texas (9) was essential in developing an adequate fungicide control program (1) where pathogen populations varied.

METHODS

Diseased seedlings were randomly collected from fields at four locations in the Yazoo-Mississippi Delta, from Yazoo City in the south to Tunica in the north. Some of the samples were taken in conjunction with field tests of hopper-box applied fungicides (8); other samples were taken from fields exhibiting mild to severe symptoms of seedling disease. In each case, a minimum of 120 diseased seedlings were collected at each year-location. Diseased sections were cut from the infected seedlings and washed in running tap water for 20 minutes and rinsed twice with distilled water. The sections were plated on 2% water agar and held at approximately 75° F for sufficient time to permit identification.

RESULTS AND DISCUSSION

The results (Table 1) indicate that *Rhizoctonia solani* Kuehn is the predominant organism involved in the seedling disease complex in the Yazoo-Mississippi Delta. In the two instances where the infection level caused by *R. solani* dropped below 60% there was marked change in only one of the other groups involved. At Clarksdale in 1960, when the loss due to *R. solani* was only 54.7%, infected seedlings colonized with *Fusarium* spp. accounted for 23.4% of the total, over twice the percentage found in 1959 and 1961. At Stoneville in 1961, infected seedlings colonized with *Pythium* spp. accounted for 19.8% of the total loss, over six times the average percentage recorded in 1959 and 1960. Weather may partly account for these apparent deviations. In 1960 at Clarksdale, an extended cool but dry period followed planting. At Stoneville in 1961, a prolonged cool and wet period followed planting.

Subsequent to identification of the fungi involved in the disease complex at Clarksdale in 1959 information was received that one-half of the seedlings sampled were from seed that had not received treatment with a recommended seed treatment fungicide (10). This may partly explain the high level of infection by *Glomerella gossypii* Edg. In all other cases, seedling samples were collected from fields planted with seed treated with a recommended seed treatment material.

This survey indicates that a chemical control program must be effective against *R. solani* and must also prevent the severe development of the other groups of fungi involved in the complex.

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Table 1. Fungi involved in the cotton seedling disease complex in the Yazoo-Mississippi Delta.

Location and year	Seedlings colonized by				
	Rhizoctonia solani	Pythium spp.	Fusarium spp.	Glomerella gossypii	Other fungi[a]
	%	%	%	%	%
Yazoo City:					
1959	71.3	3.6	11.1	9.8	4.2
1960	76.2	2.8	10.5	4.9	5.6
1961	62.3	5.4	13.1	7.7	11.5
Stoneville:					
1959	63.9	3.7	16.7	4.6	11.1
1960	70.9	2.1	5.7	13.5	7.8
1961	54.2	19.8	9.9	6.9	9.2
Clarksdale:					
1959	61.7	11.2	8.6	18.5	0
1960	54.7	3.5	23.4	11.3	7.1
1961	63.1	8.8	11.3	4.4	12.4
Tunica:					
1959	62.2	10.6	6.6	16.6	4.0
1960	63.1	5.4	20.0	5.4	6.1
1961	60.1	8.0	12.8	8.8	10.4
Mean:					
1959	64.8	7.3	10.8	12.4	4.8
1960	66.2	3.5	14.9	8.8	6.7
1961	59.9	10.5	11.8	7.0	10.8
1959-61	63.6	7.1	12.5	9.4	7.5

[a] Of frequent occurrence in this group are species of *Alternaria*, *Aspergillus*, *Penicillium*, and *Thielaviopsis*.

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SEASONAL VARIATION IN THE INCIDENCE OF RADISH YELLOWS AND EFFECT OF CROP ROTATION ON PLANT SURVIVAL AND YIELD

J. D. Wilson[1]

Abstract

Radish yellows, caused by a species of Fusarium, is most active during the period of high air and soil temperatures from about July 1 to September 15 in the latitude of north-central Ohio. In a series of plantings made from early May to late September in a plot that had been planted to radishes each of the 4 previous years, and in which the causal organism was well established, only about 20% of the initial plant stand in the first planting, which was made early in May, was lost by June 1. This loss increased to over 70% in harvests made in August and then decreased slightly to 62% in a late October harvest. In two plots planted to radishes only once in every 3 years the loss from yellows did not exceed 23.5% even in mid-summer. In a 2-year rotation the mid-summer loss was approximately 50%. The soil temperature at a depth of 8 inches varied from 50° F in early May to 80° in August, and would of course have been much higher than that at the 2- to 3-inch depth where the radishes were growing in mid-summer. Thus the seasonal curve of disease incidence followed that of soil temperature rather closely, except that the percentage of disease decreased more slowly in the fall than did the soil temperature.

INTRODUCTION

Radish yellows, caused by Fusarium oxysporum f. conglutinans (1), has become so common in many Ohio fields that it is no longer profitable to grow this crop unless a yellows resistant strain is planted. Many growers who use radishes as a part of their crop rotation sequence, or who wish to have them available to complete a list of vegetables being furnished to retail outlets, are now planting Fusarium susceptible strains in the spring and fall and one of the more resistant ones in mid- and late summer.

Studies on the control of this disease by chemical means was initiated at the Ohio Experiment Station several years ago (2, 3, 4, 5), and although a considerable degree of control was obtained in some instances, the expense involved was prohibitive for the use of most of the soil fungicides. Also, the causal organism was never eliminated completely, and as a result even low degrees of infestation immediately following chemical treatment soon built up to cause crop losses as great, or even greater in some instances, than those that occurred in untreated check plots.

An alternative to chemical treatment is crop rotation. In 1957, an experiment was started at the Muck Crops Experiment Station at Celeryville, Ohio, in which radishes were to be planted in a continuous sequence in one plot, and in 2 out of 3 years, every other year, and every third year, in other plots. By the third year (1959) the disease became relatively severe in the continuous plot and by 1960 was active enough to nearly destroy the crop in mid-summer plantings (6). The other vegetables included in this crop rotation study and used in various rotations with radish were carrot, celery, onion, lettuce and potato. Some of these are also subject to attack by various species of Fusarium, but not by the causal organism of radish yellows.

PROCEDURE

In 1961, the fifth year of the rotation experiment, a planting of radishes was made approximately every 2 weeks, beginning about May 5 and ending about September 30, to study the relationship between soil and air temperatures and the severity of disease development. These 2-week plantings were arranged for by dividing each of the 24-foot wide plots into two 12-foot sections and then making alternate plantings on the two halves. The soil and air temperatures were measured a short distance from the crop rotation plots by means of a combination soil-air thermograph, the soil tube of which was buried 8 inches beneath the soil surface, and the air temperature was determined about 4 feet above ground level. It takes from 21 to 28 days from seeding for a crop of radishes to mature, depending upon the soil and air temperatures during the growth period.

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Table 1. Seasonal variations in plant survival and yield of radishes grown in plots planted in various crop sequences during the 4 preceding years. Also, seasonal variation in soil temperature at a depth of 8 inches.

Rotation	Date of harvest										
	6/5	6/13	6/30	7/14	7/31	8/9	8/28	9/13	10/16	10/24	Averages
Percent survival											
Continuous	81	77	51	42	32	28	31	28	37	38	45
2 out of 3 years	87	87	67	56	47	39	40	47	48	52	57
Every second year	84	90	71	67	60	52	48	57	56	58	64
Every third year	92	97	90	90	83	83	77	85	79	82	86
Averages	86	88	70	64	56	50	49	54	55	58	63
Average soil temperatures for the first 3 weeks of each growth period	55	54	64	65	69	72	70	70	60	56	
Yield											
Pounds per 100 feet of row											
Continuous	9	5	5	5	5	5	11	7	9	5	7
2 out of 3 years	12	7	11	7	11	8	15	15	12	12	11
Every second year	9	11	10	13	13	11	17	18	11	10	12
Every third year	21	12	23	25	25	22	26	24	17	17	21
Averages	13	9	12	13	13	11	17	16	12	11	13

An initial stand count was made in four 100-foot rows in each plot as soon as all of the plants were up. This count was followed by two others, one in 2 weeks and the other just before the crop was to be harvested. Yields in the number of pounds of radishes per 100 feet of row were determined at each harvest. The data relative to yield and to the percentages of plant survival (final stand divided by the initial count) were later calculated for each type of crop sequence (one plot planted continuously, two for both the 2- and 3-year sequences, and three planted to radishes 2 out of 3 years). The yield and survival data are presented for the harvest rather than the planting dates since the intervals between seeding and harvest varied. Yield data are not too significant in terms of seasonal variation since the various plantings were not always harvested on the exact day when the roots were of comparable size. The survival data, on the other hand, deviate less from what could be obtained by adhering to a highly accurate schedule of harvest dates.

RESULTS AND DISCUSSION

In the spring when soil temperatures were low the first two harvests from the plot that had been planted to radishes each of the four previous years were not too different from the other sequences; but by June 30, when soil temperature had risen to 75° F, the percentage of plant survival had dropped to not much more than half that in the 3-year rotation; and by August 9 the survival was only about one-third that in the plots that were not planted to radishes during the 1959 and 1960 seasons. These relationships between seasonal soil temperatures and disease incidence with the different crop sequences are shown in the first section of Table 1 where the data are given for each planting, and are pictured graphically in Figures 1 and 2 as averages of the values for each successive pair of plantings. The failure of disease incidence to drop as sharply in the fall as did the soil temperature was probably due to two factors. First, the inoculum potential was high just as the soil began to cool, which would tend to maintain a high level of infection; and secondly, initial infection had already occurred in plantings of a given date and the disease progressed in the infected plants from that time until harvest in spite of the drop in the temperature of the soil surrounding the plants.

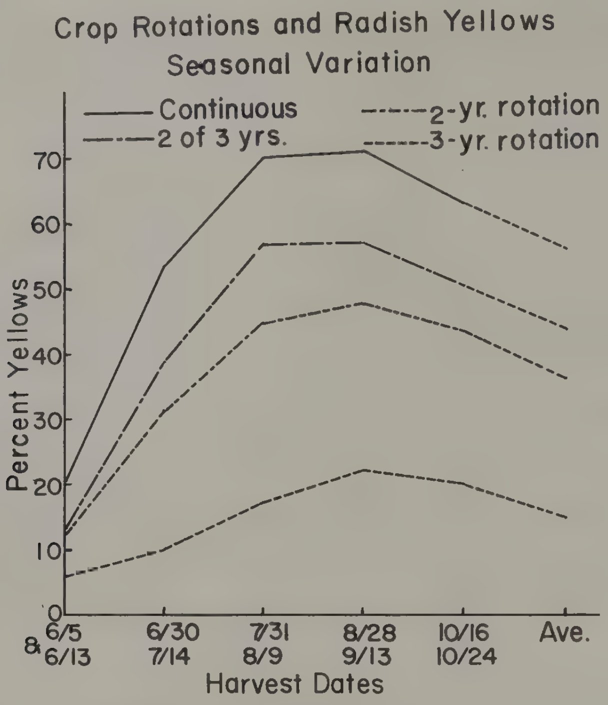

FIGURE 1. Seasonal variation in percentage of radish plants killed by radish yellows in plots planted in different crop sequences over a period of 5 years.

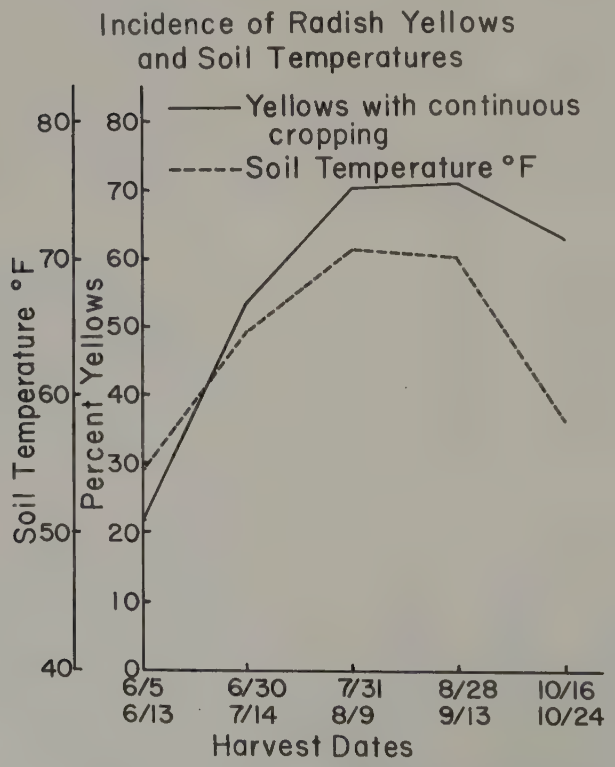

FIGURE 2. Relationship between incidence of radish yellows and seasonal variations in soil temperature.

On June 30 the yield from the continuous plot was only one-fourth that with a 3-year rotation and this ratio held for several of the harvest dates during the rest of the season. The average yield for the entire season was only about one-third as great from the "continuous" plot as from that planted to radishes only once in every 3 years. The average survival percentage, on the other hand, was approximately one-half as great with continuous cropping as with a 3-year rotation.

When every third crop was something other than radishes, and it made little difference whether it was celery, lettuce or onion, the average seasonal survival was approximately 57%, compared with 44.5 and 88.5, respectively with the continuous and 3-year rotations. This survival value was 64.2% in the plots planted to radishes during every other year. The average seasonal yields were approximately twice as great with the 2-year and 2 out of 3 years sequences as with continuous cropping, but were only 60% as great as they were when two other vegetables intervened between each crop of radishes (the 3-year rotation).

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DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, OHIO AGRICULTURAL EXPERIMENT STATION, WOOSTER

THE CAUSE OF POTATO RUSSET SCAB IN MINNESOTA[1]

M. D. Harrison and Carl J. Eide[2]

A disease of potato tubers known as russet scab or "scurf" is a serious problem in the Red River Valley potato area of Minnesota and North Dakota. It is also present in other areas of Minnesota and in other States. The symptoms of the disease (Fig. 1) consist of shallow corky reticulations forming patterns of various types on the tuber surface and giving it a rough, scurfy appearance. Often large portions of the tuber surface are affected but the damage seldom extends much deeper than the periderm.

Similar tuber blemishes have been reported by several workers (1, 2, 5, 6) and have been ascribed to several agents including Rhizoctonia (2, 5) and strains or species of actinomycetes (1). In some cases no pathogen was isolated from affected tubers (6).

Attempts to isolate a pathogen from infected tubers by dilution plates (3) and phenol treatment (4) were unsuccessful. A new technique was tested in 1961, in which russet scab lesions from surface-sterilized tubers were sectioned with a sterile razor blade and the sections placed on 3% water agar in Petri dishes. With this method a species of Streptomyces was isolated from 82% of the lesions on freshly harvested tubers with russet scab symptoms.

Fifteen isolates of the organism were tested for pathogenicity on Red Pontiac potatoes in the field and greenhouse. In the greenhouse, autoclaved soil was artificially inoculated with an aqueous suspension of inoculum prepared by growing the isolates on potato-dextrose agar in Petri dishes for 10 to 14 days then macerating them with water in a food blender. Symptoms similar to those resulting from natural infection (Fig. 2) were consistently produced on the tubers grown in inoculated soil and reisolations from sections yielded the original organism in all cases.

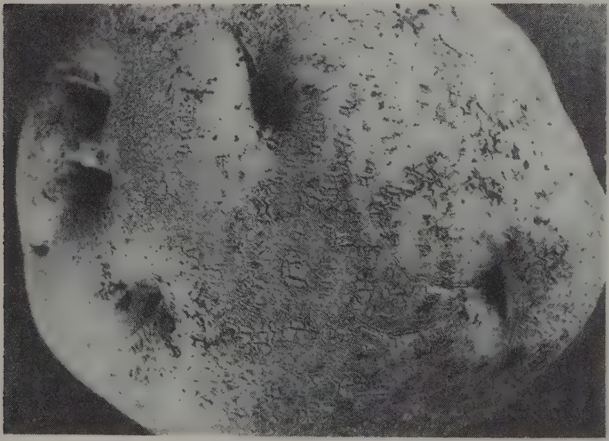

FIGURE 1. (left) Russet scab on a field-grown Red Pontiac tuber.

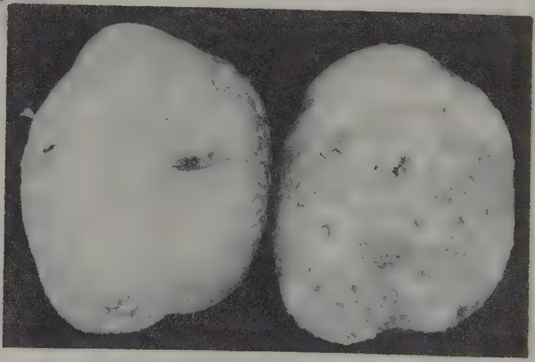

FIGURE 2. (right) Russet scab symptoms on Red Pontiac tubers grown in the greenhouse. Left, grown in naturally infested soil. Right, grown in artificially infested autoclaved soil.

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[1] Paper No. 4765, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota.

[2] The authors are, respectively, Research Fellow of Plant Pathology and Professor of Plant Pathology, University of Minnesota, St. Paul, Minnesota.

This work was supported by a grant administered by the Minnesota Potato Control Board and the Minnesota Certified Seed Potato Growers' Advisory Committee.

The cultural and morphological characteristics of the pathogen do not agree with those of any species of Streptomyces supposedly pathogenic on potato. It differs from S. scabies principally in not producing dark pigment on protein-containing media and in not producing aerial mycelium and spores on many media. Soil temperature and moisture requirements for the disease also are higher than those of common scab.

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ROCKINGHAM, A NEW TOMATO VARIETY RESISTANT TO LATE BLIGHT[1]

A. E. Rich, M. C. Richards, and E. M. Meader[2]

A new late blight resistant tomato variety called New Hampshire Surecrop[3] was released by the New Hampshire Agricultural Experiment Station in 1957. Rockingham is a new variety of higher quality than New Hampshire Surecrop.

Rockingham originated from a cross between Rockefeller Foundation Selection X907W and one of the New Hampshire breeding lines[4]. The late blight resistance is inherited from X907W, a small cherry tomato which is highly resistant to Phytophthora infestans (Mont.) d By., Tomato Race 0. Backcrosses were made to Mikado, a potato-leafed variety, and to Rutgers[4]. Rockingham was finally selected for its blight resistance, large fruit size, and quality.

Plant description: semideterminate, potato leaf, blight resistant.

Fruit description: large, red, oblate, slightly fasciated, many locules, firm, relatively free from cracking, mid-season to late (comparable to Marglobe). Fruits highly resistant but not immune to late blight.

NEW HAMPSHIRE AGRICULTURAL EXPERIMENT STATION, DURHAM

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[1] Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 284.

[2] Plant Pathologist, Mycologist, and Associate Horticulturist, respectively, New Hampshire Agricultural Experiment Station.

36.424

[3] Rich, A. E., and A. F. Yeager. 1957. New Hampshire Surecrop -- A new tomato variety highly resistant to late blight and moderately resistant to early blight. (Abst.) Phytopathology 47: 28.

[4] The crosses and selections were made by the late Dr. A. F. Yeager, formerly Head, Department of Horticulture and Horticulturist, New Hampshire Agricultural Experiment Station.

BOOK REVIEWS

"COLOR HANDBOOK OF CITRUS DISEASES," Third Edition, by Leo J. Klotz, University of California. 75 pages. 1961. Price $2.25.

The University of California has issued a third edition of its citrus disease handbook, containing several improvements that reflect the good work of its author, Leo J. Klotz.

Principally through the medium of color photographs -- most of them taken by Dr. Klotz -- this latest edition provides citrus growers and pathologists with descriptions of symptoms of 113 important citrus diseases. In addition, the handbook includes the latest control recommendations for each disease. Almost half of the 75-page volume is devoted to virus diseases. -- PAUL R. MILLER

"A MONOGRAPH OF THE WORLD SPECIES OF HYPOXYLON." by Julian H. Miller, University of Georgia Press, Athens, Georgia. xii + 158 pages, 76 plates. 1961. Price $6.50.

It is a matter of deep regret to his many friends and to his mycological colleagues in particular that Julian H. Miller, author of the book under review, did not live to see the volume published. He passed away on March 25, 1961, just before production on the book began. It represents the culmination of studies commenced about 1924 and was planned as the first of a series of monographic studies of the Xylariaceae. The genus Xylaria was nearly completed and publication could have followed very shortly.

The many species of the genus Hypoxylon occur on woody substrates, mostly on the dicotyledons; there are surprisingly few on the monocotyledons or conifers. They are at best weak parasites only. Initial infections may occur on a living tree, but it will usually be one injured by fire, drought, or other agencies causing mechanical injuries or a general weakening of the host. To this extent some of the species such as H. atropunctatum, so well illustrated in the unnumbered plate, are of some economic concern.

In discussing the history of the genus Miller points out that the genus name was first used by Bulliard in 1791, but Fries in his Systema Mycologicum continued Persoon's usage of the all-inclusive Sphaeria and did not accept Hypoxylon as a generic entity until 1849 in his Summa Vegetabilium Scandinaviae. As there circumscribed, the genus has been accepted by Miller and most other taxonomists concerned with the genus since that time. Unfortunately the name is not valid under the present code of botanical nomenclature and steps must therefore be taken to conserve it. It is very much to be hoped that it will not be considered necessary to transfer the large number of species involved to some otherwise long forgotten generic name.

The author studied, insofar as it was possible to obtain types or authentic material, all species described in the genus as well as those assigned originally to other genera, but which belong within the present concept of Hypoxylon. As part of this study attention was given to variations brought about by external factors such as climate and host reactions. It was found possible to classify available material into 96 species and 24 varieties. Several hundred names were placed in synonymy. For example, the common and widespread species H. rubiginosum finds itself with 56 synonyms. Six new species and seven new varieties are described.

The genus is divided into four sections, three of which are newly described. The sections are distinguished on the basis of variations in the stromata and ostiola. Adequate keys are provided to characterize sections and species in each section. Specific descriptions are adequate and uniform in presentation. With each species description pertinent synonymy, geographical distribution, and a list of important specimens used in studying each species are included. It will be obvious that not all of the specimens used in the study could be cited, since the author had available, in addition to his own extensive collections made over many years, large numbers on loan from herbaria around the world. For example, he notes that he had 8000 specimens from the National Fungus Collections alone.

An outstanding feature of the book are the 76 plates illustrating macroscopic and microscopic characters of most of the species involved. In fact only six species and three varieties are not illustrated in at least one of these categories. 119 excluded species are briefly discussed and their proper classification indicated. Finally, a comprehensive fungus index is provided.

This work is an important contribution to taxonomic mycology and will long remain a basic source for information on this important fungus. -- JOHN A. STEVENSON

THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 46 Number 3

March 15, 1962

Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to twelve double-spaced typed pages, including tables, graphs, and photographs. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases.

Paul R. Miller

Manuscripts for and correspondence about this publication
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PLANT DISEASE REPORTER
Epidemiology Investigations, Crops Protection Research Branch
Plant Industry Station, Beltsville, Maryland

THE PLANT DISEASE REPORTER

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CONTENTS

1. Comparative effectiveness of some equipment and methods of measuring low concentrations of ozone
GUSTAVE SILBER 137

2. Daily fluctuation of phytotoxic air pollutants in three New Jersey communities as influenced by certain meteorological parameters
IDA A. LEONE, et al. 140

3. Effect of irrigation method and frequency on development of Phytophthora infestans on potatoes under arid conditions
J. ROTEM, et al. 145

4. Pear decline on Ikaria Island
A. G. PLAKIDAS 150

5. Control of Fusarium wilt of tomato with Vorlex
P. A. YOUNG 151

6. A survey of carnation diseases in south Sweden
GOSTA I. NILSSON 152

7. Attrition of a saguaro forest
STANLEY M. ALCORN and CURTIS MAY 156

8. Apple scab II. Effect of serial applications of fungicides on leaf lesions on previously unsprayed trees
J. W. HEUBERGER and R. K. JONES 159

9. Effect of repeated applications of dodine and of captan on apple scab foliage lesions
J. J. ALBERT and F. H. LEWIS 163

10. Bacterial blight of cotton in Arkansas
B. E. McCUTCHEN and N. D. FULTON 168

11. The inhibitory effect of 2-chloroethyltrimethylammonium chloride treatment on tobacco mosaic virus (TMV) multiplication
T. E. RAWLINS 170

12. New findings of plant-parasitic nematodes in Peru
J. N. SASSER, et al. 171

13. A disease of snow-in-summer, cypress euphorbia, and Corsican sandwort caused by a species of Rhizoctonia
LESTER P. NICHOLS 172

14. Stunt nematodes and tobacco in Gujarat, India
G. J. PATEL, et al. 173

15. Bacterial spot disease of tomatoes and peppers in Israel
ZAFRIRA VOLCANI 175

16. Current-season and tuber-perpetuated symptoms of potato curly top
GOLDEN L. STOKER and ORSON S. CANNON 176

17. Injury to soybeans caused by Pratylenchus alleni
VIRGINIA R. FERRIS and R. L. BERNARD 181

18. Occurrence of citrus sour rot in Louisiana
J. B. SINCLAIR, et al. 185

19. The effect of varying degrees of root knot infestation on winter injury to the perennial Caryopteris
J. D. WILSON and ORVE K. HEDDEN 186

20. Dutch elm disease in Kansas in 1961
C. L. KRAMER and HUGH E. THOMPSON 189

21. The influence of time of planting and runner removal on the incidence of Verticillium wilt in strawberry
J. N. MOORE and E. H. VARNEY 190

22. A new survival record for certain fungi on agar under a mineral oil seal
G. L. BARNES 192

23. Seasonal inoculum dispersal and white pine stump invasion by Fomes annosus
W. J. STAMBAUGH, et al. 194

24. A root observation box and techniques for studying infection and pathogenesis of roots by nematodes
NORMAN A. MINTON 199

25. Leaf spot on Rangpur lime and its control
JULIUS L. HEINIS 200

26. Rhynchosporium leaf scald of rice in Guatemala
EUGENIO SCHIEBER 202

27. Fusarium root rot of maize in Israel
A. JOFFE 203

28. Powdery mildew of safflower
ARTHUR H. McCAIN 203

29. Book Review (The genus Tilletia)
JOHN A. STEVENSON 204

30. Book Review (Fungicides in agriculture and horticulture)
HILDE McGRATH 205

31. Announcement 205

COMPARATIVE EFFECTIVENESS OF SOME EQUIPMENT AND METHODS OF MEASURING LOW CONCENTRATIONS OF OZONE

Gustave Silber[1]

Abstract

Test tube-type gas impingers fitted with an intake of 1-mm capillary bore tubing or a fritted glass cylinder dispersion tube were compared for their effectiveness of ozone recovery. Impingers of 1-mm capillary tube recovered 14 to 30% more ozone than did fritted glass-tube impingers. Ozone concentration of atmosphere was secured simultaneously by a commercially available ozone meter and by chemical analysis, using neutral buffered potassium iodide solution as the collecting medium in blackened test tube impingers fitted with a 1-mm capillary bore intake. Meter readings were lower and ranged from 55 to almost 83% of values determined by chemical analysis.

INTRODUCTION

Weather fleck of tobacco, caused by elevated ozone concentrations in the atmosphere, is a serious problem in the production of quality cigar-wrapper tobacco (2, 3, 5). In greenhouse and field studies of weather fleck it was necessary to monitor the ozone concentration of the atmosphere within a growth chamber and the oxidant concentration of the atmosphere in the field.

A wide variety of glassware, techniques and equipment is available and has been utilized for gas analyses. This is a report of the results obtained in the course of investigations concerned with the suitability of different equipment for ozone or oxidant determinations.

MATERIALS AND METHODS

Ozone concentration of atmosphere was determined by the neutral potassium iodide procedure (1). Air was automatically and continuously monitored for ozone concentration by a Mast Development Company ozone meter[2] (Fig. 1). The equipment used to measure the volume of air being sampled was described previously (4).

Many gas scrubbers are constructed of fritted glass. Such equipment is suitable for the determination of some gases but has been reported to give low values for ozone. Blackened test tubes (25 x 200 mm) fitted with neoprene stoppers and 1-mm capillary bore tubing are a satisfactory and inexpensive type of gas impinger in lieu of commercially available midget impingers (Fig. 2). Smaller bubbles of the sampled gas are dispersed to the collecting medium if the discharge end of the capillary tube is ground to an acute angle.

Effectiveness of the capillary bore impinger and that of a fritted glass cylinder gas scrubber were compared on ozone-containing atmospheres within a growth chamber modified for fumigation of plants.

Ozone was generated from cylinder oxygen passed over glass-encased multiple silent-discharge tubes operating at high voltage. The generated ozone was diluted with a constant flow of carbon-filtered air and fed into the growth chamber from which the atmosphere was withdrawn for sampling. Simultaneous air samples were drawn through 25 ml of neutral 1% potassium iodide solution in separate blackened test tubes fitted with 1-mm capillary glass tubing or a coarse fritted-cylinder gas dispersion tube. The air was sampled at a rate of 1 liter per minute in all tests. Twenty liters of atmosphere were sampled in the majority of cases but in a few instances as much as 60 and as little as 10 liters of air were sampled.

The Mast ozone meter, which continuously monitors ozone concentration, employs a medium containing potassium iodide and measures ozone concentration coulombmetrically. As an ozone-containing atmosphere is metered into the microcoulomb sensing unit, it reacts with the potassium iodide-containing solution being continually metered into the sensor. The electron flow involved in the oxidation-reduction of potassium iodide is transmitted to an external circuit where it is either observed on a microammeter or measured and recorded on calibrated graph paper. The ozone levels in the laboratory were measured simultaneously by means of the Mast ozone meter and spectrophotometrically by using a neutral potassium iodide solution as the collecting medium. Oxidant levels in the field have also been measured simultaneously by these two means.

---

[1] Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

[2] Use of a name of a specific commercial product does not constitute endorsement by the United States Department of Agriculture.

RESULTS AND CONCLUSIONS

Impingers of capillary tubing gave greater ozone recovery than did impingers of fritted gas-dispersion tubes (Table 1).

Table 1. Comparative ozone recovery by gas absorbers fitted with intakes of 1-mm capillary tubing or fritted gas-dispersion tubes.

Sample volume (liters)	Ozone concentration (pphm)		Percent
	Fritted tube	Capillary tube	Fritted:Capillary
10	81.3	97.7	83.2
20	41.0	50.4	81.1
20	42.5	60.6	70.1
20	49.7	65.3	76.1
20	52.0	62.0	83.9
20	54.3	68.8	78.9
20	71.9	90.5	79.4
60[a]	3.8	4.4	86.4

[a] Ambient atmosphere sample.

The fritted gas-cylinder dispersion tubes used as impingers reduced the recovery of ozone approximately 14 to 30% below that obtained by the capillary tube-type impingers. These results confirm the assertion that ozone is decomposed by contact with fritted glass (6).

Comparative ozone determinations by the Mast ozone meter using the calibration supplied by the manufacturer and by gas impingers fitted with capillary tubing with neutral potassium iodide solution as the collecting medium are shown in Table 2.

Table 2. Comparative ozone concentrations as measured coulombmetrically and spectrophotometrically.

Sample volume (liters)	Ozone concentration (pphm)		Percent
	Spectrophotometric	Coulombmetric (ozone meter)	Meter: Spectrophotometric
10	117.2	70-74	61.4
10	97.7	72-75	75.2
20	40.8	31	76.0
20	42.6	32-34	77.5
20	13.8	6.8-8.4	55.1
20	10.2	6.8-8.4	74.5
20	12.2	6.8-8.4	62.3
20	13.1	7.8-11.2	72.5
20	10.2	8.0	78.4
20	9.7	7.0	72.2
20	9.4	7.5	79.8
20	14.8	9.8	66.2
20	12.5	10.0	80.0
20	13.8	9.8	71.0
56[a]	2.8	2.1-2.4	80.3
56[a]	2.9	2.4	82.8

[a] Ambient atmosphere sample.

The two methods of measuring ozone agree poorly. There is, however, high correlation between the two since values by the spectrophotometric technique invariably are higher than those secured by the ozone meter. Meter readings range from 55 to almost 83% of values found by the more routine chemical analysis of the atmosphere by using buffered neutral potassium iodide solution as the collecting medium. The coulombmetric method of measuring ozone concentration offers the advantage of continuous monitoring, but such measurements for critical work should be related to determinations based on chemical analyses.

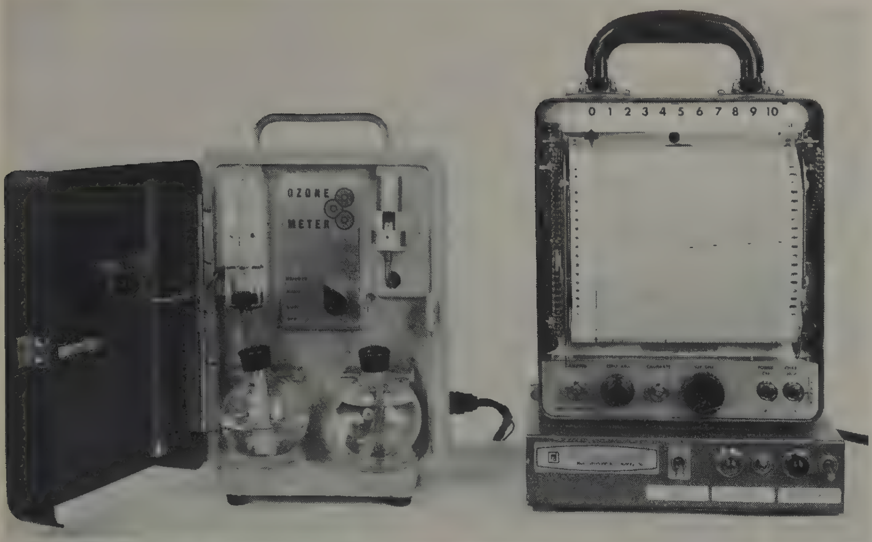

FIGURE 1. Mast Development Company, Inc. ozone meter. Right -- Continuous recorder. Left -- Ozone meter with microcoulomb sensor.

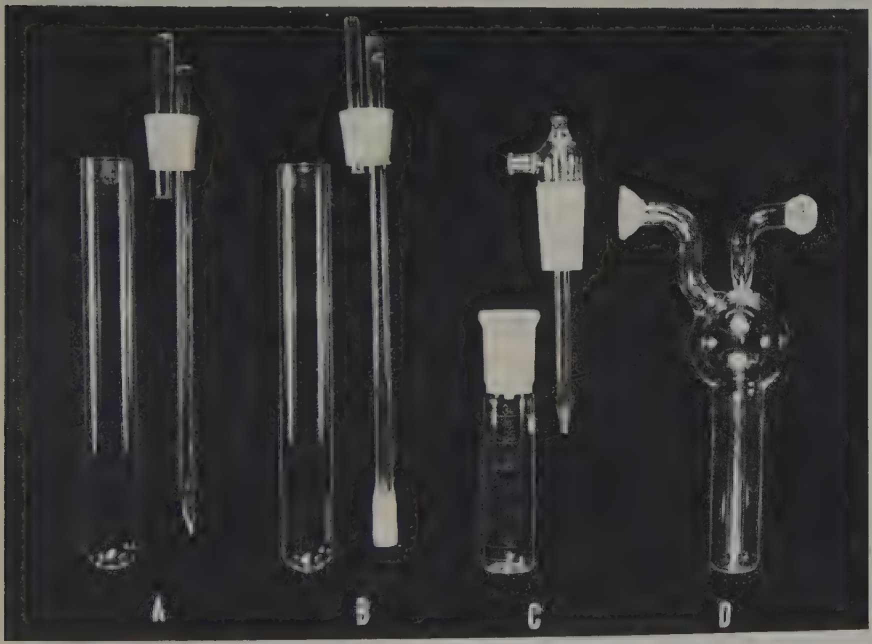

FIGURE 2. Types of gas impingers. A -- Test tube with capillary bore intake. B -- Test tube with fritted glass cylinder intake. C and D -- Commercially available midget impingers.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

DAILY FLUCTUATION OF PHYTOTOXIC AIR POLLUTANTS IN THREE NEW JERSEY COMMUNITIES AS INFLUENCED BY CERTAIN METEOROLOGICAL PARAMETERS[1]

Ida A. Leone, Eileen Brennan, and Robert H. Daines[2]

Summary

Concentrations of three common phytotoxic gases, sulfur dioxide, oxidant, and nitrogen dioxide, were measured daily in three New Jersey farming communities during September and October 1960. The extent of air pollution was evaluated in each community and the data plotted against three meteorological parameters: barometric pressure, wind speed, and visibility. Results showed a direct relationship of pollution load to barometric pressure, and an inverse relationship to wind speed and visibility. Data such as these may provide a basis for an air pollution forecasting system that would indicate the appropriate time for taking control measures.

INTRODUCTION

Many investigators have shown in the past decade that certain air pollutants can cause injury to vegetation (1, 4, 6, 7, 9, 10, 13, 14, 20). While early work was concerned with symptomatology, species susceptibility, and other descriptive phases, more recent investigation has been directed toward control of the air pollution problem. In the case of primary contaminants, or those arising from a specific industry or group of industries, engineering technology, accelerated by or compelled by legislation, has done much toward decreasing injurious effects from fluoride, chloride, and other phytotoxic acid gases. Contaminants such as ozone, oxides of nitrogen, peroxides, and other oxidants resulting from an expanded urban population and increased vehicular traffic cannot, however, be easily controlled at their source. While the enforced use of "blow-by" devices on automobiles will cut down the contribution to the general pollution load by the automotive industry, there still remains the problem of home heating and industrial furnaces which emit products that, although not especially harmful per se, may initiate photochemical reactions yielding harmful products (17). Two present methods of dealing with the problem of control are the selection of resistant varieties (5) and the application of deterrent sprays (2, 3, 8, 16, 18, 19). In order to use these control methods effectively, it is important to identify, and, if possible, to measure the common phytotoxic air pollutants.

METHODS[3]

Air sampling apparatus was installed in three farming communities in New Jersey. Two of the communities had repeatedly reported air pollution damage to crops: Carlstadt, which is located on the fringe of a heavily industrialized metropolitan area; and Bordentown, which has a more rural location in the west-central part of New Jersey. The third station was the farm at the New Jersey Agricultural Experiment Station in New Brunswick. Initially three easily detected pollutants, sulfur dioxide, nitrogen dioxide, and oxidants, were sampled at a specified time daily at each site during October and November.

Sulfur dioxide (SO_2) and total oxidant (reported as ozone) were sampled by scrubbing air through appropriate absorbing solutions in modified Greenburg Smith impingers, whereas nitrogen dioxide (NO_2) was collected in a fritted glass bubbler. The sampling was done during the 4-hour period from 11:00 a.m. to 3:00 p.m., theoretically the period of maximum pollution concentration. Samples were stored in polyethylene bottles and periodically taken to the laboratory for chemical analysis. Sulfur dioxide was determined by the West and Gaeke colorimetric method (11), with 0.1M sodium tetrachloromercurate employed as the absorbing solution. Total oxidant was determined by a modified Salzman Method (11) using 1% potassium iodide in 0.01N sodium hydroxide solution as the absorbent. Results were expressed as ppm ozone. The NO_2 procedure was that of Jacobs and Hochheiser (11) using 0.1N sodium hydroxide as the absorbent. The air was sampled at a rate of 5 cubic feet per hour, which for a 4-hour sampling period represented a total volume of 566.4 liters of air per sample. The resulting daily concentration of each pollutant for all three localities was plotted.

---

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[3] Acknowledgment is expressed to the New Jersey State Department of Health for assistance in devising the sampling methods.

RESULTS AND DISCUSSION

Figure 1 presents the daily average SO_2 content for the 4-hour sampling period in each community. Sulfur dioxide concentration showed a wide daily fluctuation ranging from 0 to nearly 0.3 ppm. Carlstadt was by far the most highly polluted community with respect to SO_2 content. It is interesting that, regardless of magnitude, the peaks for the three communities coincided on the same days. Figure 2 shows the oxidant content of the three communities. As was the case with SO_2, oxidant varied from 0 to well over 0.1 ppm. Again Carlstadt air showed the highest pollutant content, with New Brunswick and Bordentown similar to each other at a much lower concentration. Again, peaks for the three communities coincided on the same days. In the case of oxidant, data on the occurrence of tobacco fleck in the greenhouse at New Brunswick were obtained concurrently with the daily pollutant analyses and this information incorporated with the pollutant data. It appeared that every time the oxidant content exceeded 0.035 ppm there was an incidence of tobacco fleck. Figure 3 indicates the NO_2 concentration of the three communities. As did the other two pollutants, NO_2 varied from 0 to nearly 0.3 ppm. Again Carlstadt showed the highest degree of pollution, New Brunswick an intermediate amount, and Bordentown the lowest, and again peaks in concentration for the three communities coincided. NO_2 is not injurious to plants in the concentrations in which it is found in the atmosphere; it is, however, a constituent of atmospheric reactions producing ozone and other phytotoxic oxidants.

Data for October and November were selected for presentation since these are the months during which most of the complaints from growers of spinach and fall truck crops have been received. The fall season is also noted for meteorological conditions conducive to air stagnation (12). When pollutants are emitted into the air from industrial operations or are created by atmospheric reactions, stable air tends to prevent their dilution or dispersion into the atmosphere and they are thus trapped near ground level where they can exert their deleterious effects on living as well as nonliving things. Meteorological parameters associated with air stability are barometric pressure, wind speed and direction, visibility, and the presence of an abnormal temperature gradient in the atmosphere whereby an inversion layer or so-called "lid" of warm air over relatively cooler air prevents the normal upward flow of the air.

To determine the relationship of these meteorological phenomena to phytotoxic pollutants, daily observations of barometric pressure[4], wind speed[4], and visibility[5] were obtained, for the same hours as the sampling period, from a weather station near the Carlstadt pollutant sampling site. These data were plotted on a graph which was superimposed on a graph of the Carlstadt pollutant data. In August 1960, the U. S. Public Health Department at Cincinnati established an alarm system whereby interested parties can be alerted when regional meteorological conditions are such that they constitute an air pollution situation serious enough to endanger human health. An alert of this type was sounded during the testing period on November 14.

Figure 4 shows the direct relationship of the average barometric pressure for the 4-hour sampling period to the average SO_2 concentration in Carlstadt. Whenever the SO_2 content was at a peak, barometric pressure showed a corresponding high point. This relationship was especially prominent during the alert period of November 14-16 when a sustained high pressure condition resulted in a tremendously high SO_2 concentration. Figure 5 presents the same pollutant data for Carlstadt with the daily wind speed and direction superimposed. Wind speed showed an inverse relationship to SO_2 concentration. Whenever the wind speed was high enough to cause dilution, pollution was low, and, correspondingly, when the wind speed reached a very low value (2 mph or less) there was an increase in SO_2 concentration. This relationship was graphically illustrated on November 14. On those days characterized by low wind speed and high pollution incidence the wind was generally from south to southwest. Two hypotheses are put forth for this phenomenon. In the first place, the anticyclonic condition leading to diminished wind velocity or air stagnation is generally characterized by winds from a southwest direction. Secondly, the presence of one of the world's most highly industrialized areas to the southwest of Carlstadt may also be responsible as a source for some of this pollution. Figure 6 shows the visibility data superimposed on SO_2 pollution data in Carlstadt. Visibility bears the same inverse relationship to pollution as does wind speed, that is, when visibility is low pol-

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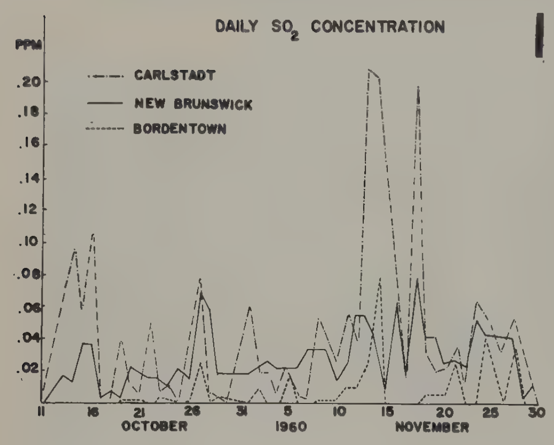
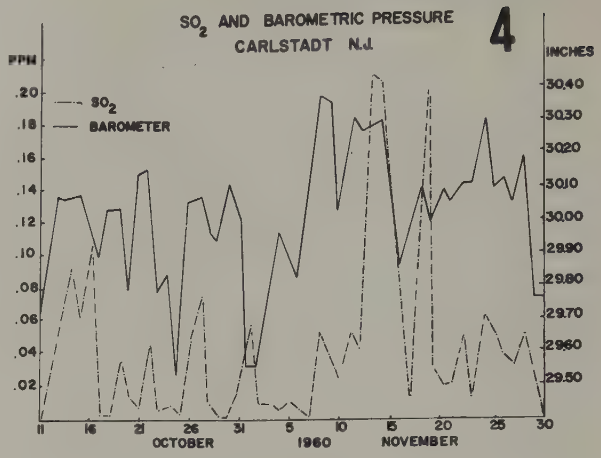
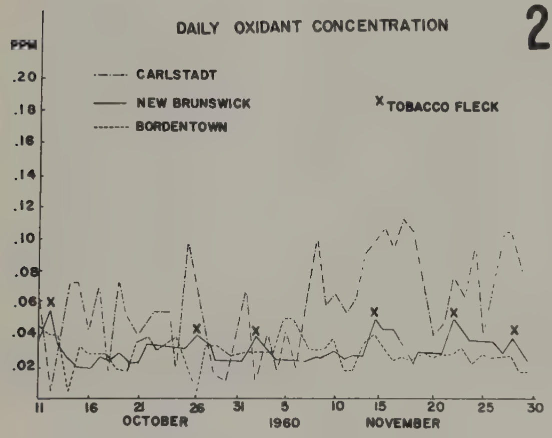
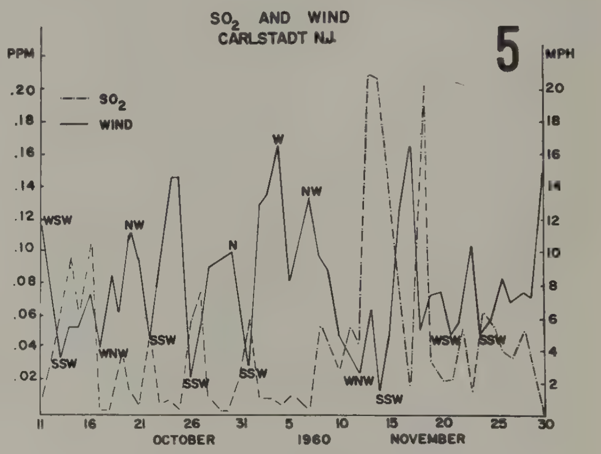
[4] Daily observations of barometric pressure and wind speed were obtained through the courtesy of Reuben Wasser of the Public Service Electric and Gas Company Testing Laboratory, Maplewood, New Jersey.

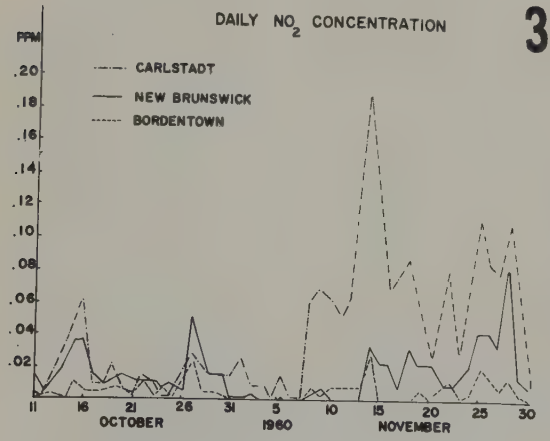
[5] Daily observations of visibility were obtained from the Newark, New Jersey Station of the U. S. Weather Bureau.











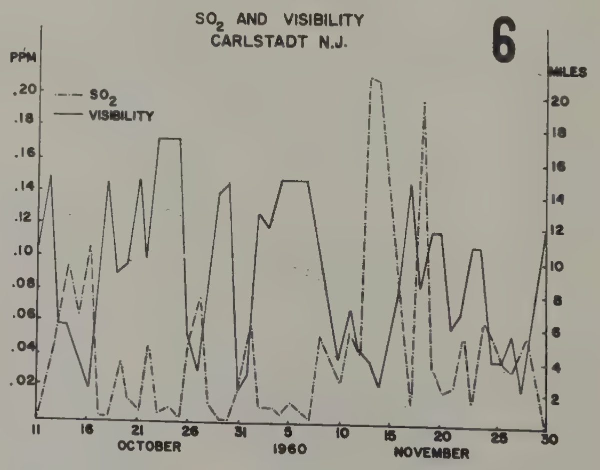


FIGURE 1. Daily concentration of sulfur dioxide in the air in Carlstadt, New Brunswick and Bordentown during October and November 1960.

FIGURE 2. Daily oxidant concentration in the air in Carlstadt, New Brunswick and Bordentown during October and November 1960, and the incidence of tobacco fleck in New Brunswick.

FIGURE 3. Daily nitrogen concentration in the air in Carlstadt, New Brunswick and Bordentown during October and November 1960.

FIGURE 4. Relationship of barometric pressure to sulfur dioxide concentration in Carlstadt.

FIGURE 5. Relationship of wind speed and direction to sulfur dioxide concentration in Carlstadt.

FIGURE 6. Relationship of visibility to sulfur dioxide concentration in Carlstadt.

lution is high, and vice versa. Lowered visibility is a result of pollution rather than a factor in its accumulation. Its effectiveness as an index of pollution is, however, as valid as the factors enhancing pollution. Simple correlation coefficients calculated numerically from these data corroborated the graphic relationships of meteorological phenomena to pollutant concentrations.

Investigations of a similar nature were made by Niemeyer (15) of the U. S. Weather Bureau. Examining the air quality data for periods in which the weather was monitored, he found that highest loadings of particulate matter occurred in periods during which the following criteria for the sounding of a National Air Pollution Alert were met: 1) Surface winds less than 8 knots. 2) Winds at no level below 500 mb (approximately 18, 000/ft.) greater than 25 knots. 3) Subsidence, the slow sinking or settling of air from aloft, below 600 mb (approximately 14, 000 feet). 4) Simultaneous occurrence of the above with the forecast continuance of these conditions for 36 hours or more. Niemeyer points out that air pollution episodes at Donora, Greater London, and other places suggest that low wind speed, variable wind direction, pronounced stability, and fog were characteristic of these episodes. Conditions persisted several days and were associated with quasi-stationary anticyclones or high pressure areas.

In conclusion, air sampling investigations have indicated that phytotoxic pollutants have been found frequently in concentration high enough to cause injury to vegetation in the air of so-called rural communities in New Jersey. It has also been demonstrated that the magnitude of these pollutants is dependent upon certain meteorological phenomena. When conditions of air stability are such that we have a temperature inversion characterized by high barometric pressure and low wind speed, atmospheric pollutants are apt to concentrate near ground level, often giving rise to a haze which lowers visibility. It is our belief that forecasts of the latter conditions coupled with measurements of air quality, could provide a basis for applying pollution controls which, in the case of vegetation, might be the use of protective sprays.

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EFFECT OF IRRIGATION METHOD AND FREQUENCY ON DEVELOPMENT OF PHYTOPHTHORA INFESTANS ON POTATOES UNDER ARID CONDITIONS[1]

J. Rotem[2], J. Palti[3], and E. Rawitz[4]

Abstract

The effect of frequency, method and rate of irrigation on Phytophthora blight development on potatoes was studied in a field trial in the autumn of 1959 in the arid northern Negev district of Israel.

The disease developed profusely under overhead irrigation, but was virtually absent from plots under furrow irrigation. The blight was much more severe on plots receiving 8-11 overhead irrigations at 7 to 10 day intervals than on plots receiving 3-4 such irrigations at 21 to 28 day intervals.

The rate of irrigation, whether extending over 3 to 4 hours at each application or (for the same amount of water) over 10 to 12 hours, had little effect on blight development.

The number of nightly hours of dew did not differ appreciably under overhead or furrow irrigation. Prevention of dew, by nightly covering plots with cloth canopies, failed to affect blight development.

INTRODUCTION

Phytophthora infestans frequently attacks irrigated potato crops in the central and western Negev, an arid part of Israel with 150 to 250 mm of winter rainfall.

When the disease appeared on the plots of a potato irrigation experiment, carried out at Gilat (northern Negev) by the Irrigation Division of the National and University Institute of Agriculture, a rare opportunity presented itself to determine the effect of various modes, rates and frequencies of irrigation on its development under conditions of little rain but fairly high dew.

METHODS

Potatoes of the Up-to-date variety were sown on loess soil at the Gilat Experimental Farm, in late August 1959.

Irrigation Treatments: Overhead and furrow irrigation plots were sown in separate randomized blocks, the former were straight, the latter followed contour lines. Overhead irrigation plots were replicated four times, each replicate measuring 24 x 24 meters; disease incidence and yield were measured only for the inner square of 12 x 12 meters. Furrow irrigation plots, replicated three times, had three rows, each 100 meters long. The following irrigation treatments were tested:

No. of series	Interval between irrigations (in days)	Total no. of irrigations
1	28	3
2	21	4
3[a]	14	6
4	10	8
5	7	11

[a] Applied as overhead irrigation only.

Water requirements were determined by gravimetric soil moisture determinations 1 day before irrigation. The amount of water actually applied was measured by water meters mounted on the irrigation pipes supplying each plot.

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[1] Publication of the National and University Institute of Agriculture, Rehovot, Israel. 1961 Series, No. 438-E.

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Two rates of water application by sprinkling were tested: 12 mm (1/2 inch) (high rate) and 4 mm (1/6 inch) (low rate) per hour. Irrigation at the former rate lasted 3 to 4 hours, at the latter rate 10 to 12 hours each time. The sprinklers were mounted on risers 50 cm high, and placed in a square, 12 x 12 meters pattern. Sprinkler plots were irrigated at night, and the furrow plots during the day.

Meteorological Records: Relative humidity was measured by hydrographs placed 30 cm above the soil surface in both furrow and overhead irrigation plots receiving treatment No. 5; temperature by a single thermograph similarly placed only in the plot under overhead irrigation.

Rain was recorded by standard rain gauges.

Dew intensity was not measured, but the duration of dew cover was recorded by means of a dew meter of the Taylor type.

The effect of dew on disease development was determined by preventing dew formation as described by Duvdevani, Reichert and Palti (2). Cloth canopies were drawn every night, from dusk to early morning, over four replicate plots, each measuring 4 x 4 meters. These plots were located within the larger plots receiving the wettest irrigation treatment, that is, at shortest intervals (7 days) and at the slowest rate (10 to 12 hours).

Disease Measurement and Field Records: Development of *Phytophthora infestans* was appraised by a scale, proposed by Littauer, Palti and Moeller (4), comprising seven categories of severity, from no disease (0 marks) to very severe incidence (8 marks), when plants have largely dried up owing to disease effect. Twenty-five plants per plot were individually appraised by this method, and the mean number of marks assigned to them was taken to reflect disease incidence in the plot. Three such appraisals were carried out, beginning 9 days after disease appearance, on November 18, 25 and December 20.

When the crop was lifted, yields were recorded separately on each plot; the incidence of blight on tubers was examined in detail on the plots receiving the most frequent overhead and furrow irrigations.

RESULTS

Meteorological Conditions During the Growing Period

Relative Humidity and Temperature: Measurements of relative humidity on plots receiving treatment No. 5 showed that considerable differences, between plots irrigated by furrows or sprinkling, existed only during the actual 3 to 4 hours of overhead irrigation and subsequent 2 to 3 hours. In the daytime, relative humidity usually fell to 37 to 50%, rising at night to 85 to 87%. Daily relative humidity and temperature, as expressed by the hourly mean for the period October 10 to November 21, 1959, is presented in Figure 1.

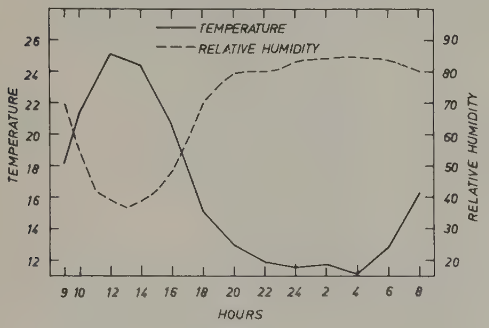

FIGURE 1. Daily relative humidity and temperature, expressed by the hourly mean for the period October 10 to November 21, 1959.

Rainfall: The experimental period was characterized by unusually low rainfall, even for the arid northern Negev. Rain fell on October 18 and 19, November 29, and 30, and December 4, 8, and 26. Of the 7 rainy days only 2 occurred before disease appearance. The total amount of rainfall during the period, 28.6 mm, was negligible compared with the amount applied by irrigation. No rain fell for about 20 days preceding and following the first disease record (November 9).

Dew: The number of hours in which dew was recorded, between October 13 and December 4, 1959, is indicated in Table 1. The number of dew hours per night was very high; dew occurred almost every night covered by the measurements, and the mean duration of dew on the sprinkler irrigated plots exceeded that on the furrow irrigated plots only slightly.

Table 1. Duration of dew on furrow and sprinkler irrigated plots.

Period of measurement	No. of nights on which dew was measured	No. of dew nights	Mean number of hours (per night) in which dew was present: overhead	furrow
Oct. 13-15	2	2	12.15	10.25
Oct. 16-31	15	15	11.45	10.30
Nov. 1-15	9	9	12	12.30
Nov. 16-30	11	11	12.35	11.20
Dec. 1-4	4	3	8.30	8.10
Total	41	40	Mean: 11.25	10.43

Incidence and Yield

Overhead and Furrow Irrigation: The first disease symptoms were recorded on November 9, 1959, about 10 weeks after the crop was sown. Subsequent disease development under the various irrigation treatments, as determined by estimations carried out on November 18 and 25 and on December 20, and yield data are presented in Figure 2.

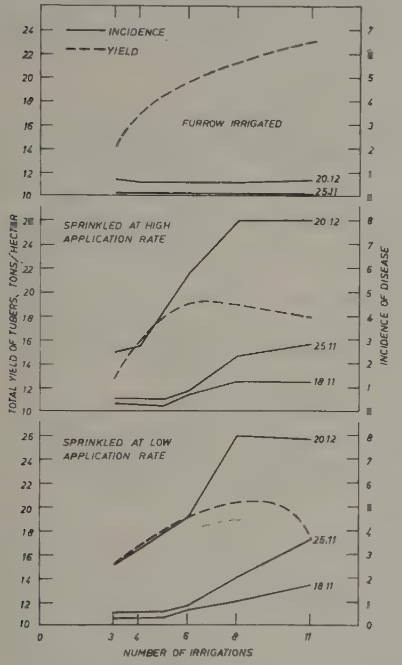

FIGURE 2. Disease development and yield under furrow irrigation and high and low sprinkler irrigation. Estimations made on November 18, 25 and December 20.

Phytophthora development in all furrow irrigated plots, regardless of frequency of irrigation, was extremely limited and usually did not exceed a few isolated lesions. On the other hand, disease incidence in overhead irrigated plots was marked, and roughly proportional to the number of irrigations applied. Thus plants that received three or four irrigations remained moderately infected (2.5 to 3.2 marks at the final estimation), while the corresponding marks for plots receiving 6, 8, or 11 irrigations were 4.5 to 5.7, 8 and 8, respectively.

A comparison between irrigation applied at a higher rate (12 mm per hour over 3 to 4 hours) and that applied at a lower rate (4 mm per hour over 10 to 12 hours) showed no noteworthy differences in disease development, regardless of frequency of irrigation.

Yield measurements showed that fewer (3-4) irrigations always produced lower yields, no matter what the mode or rate of irrigation. Six irrigations improved yields to the level of 18 to 20 tons/hectare, again regardless of the irrigation method; there were also no marked differences in yield between plots receiving 8 irrigations by furrows or by overhead application, although a slight reduction of yield occurred where overhead irrigation was applied at the high rate. A significant difference in yield was only found when 11 irrigations were applied; here plots under furrow irrigation yielded 23 tons/hectare as compared with 17 tons/hectare for plots under overhead irrigation.

In considering the above yield data it must be borne in mind that the disease affected the plants at a relatively late stage of their growth. Only pronounced differences in disease development could therefore be expected to result in significant yield differences.

The incidence of blight on tubers was too slight to permit drawing conclusions regarding the effect of various irrigation treatments.

Effect of Dew: Table 2 presents blight estimates on plants, both exposed and protected from dew, in plots that received 11 applications of either furrow or overhead irrigation. It is evident that the prevention of dew failed to affect disease development.

DISCUSSION AND CONCLUSIONS

Literature references to the effect of various irrigation methods on plant disease development are still relatively scarce.

Table 2. Phytophthora blight development on plants exposed to or protected from dew.

Date of blight estimation	Mean no. of marks on blight estimation scale (0=no disease, 8=complete defoliation)			
	Overhead irrigation		Furrow irrigation	
	without dew	with dew	without dew	with dew
Nov. 25, 1959	3.2	3.4	0-0.1	0-0.1
Dec. 20, 1959	7	8	0.5-1	0.5-1

Field observations by Menzies (5) indicated that in the dry parts of Washington State, mint rust (Puccinia menthae) developed early and strongly only on fields under overhead irrigation but not on those under furrow irrigation. Similar observations were made by Palti and Stettiner (7) concerning development of Cercospora leaf spot (C. personata) on peanuts in the dry Hula Valley of Israel.

Experimental comparisons were made in the Coastal Plain of Israel with regard to cucumber downy mildew and banana fruit rot. Duvdevani, Reichert and Palti (2) found that overhead irrigation favored Peronoplasmopara cubensis on cucumbers when dew was absent, but on plants exposed to dew the disease developed under both overhead and furrow irrigation. Rotem and Chorin (8) showed that furrow irrigation minimized the development of Dothiorella gregaria on bananas, while overhead irrigation favored the fungus by prolonged moistening of the flower attached to the fruit.

Menzies (6) studied the effect of irrigation on the development of bacterial diseases of beans (especially Xanthomonas phaseoli) under extremely dry conditions in Washington. Applications by sprinklers over very long periods (up to 30 hours) promoted development of these diseases, as compared with furrow irrigation. It was further found that moistening leaves before inoculation had a more pronounced effect on disease incidence than the actual period of overhead irrigation. He also considered reduction of atmospheric temperature levels and spread of inoculum by splashing to favor the disease.

Keeping plants in moist chambers was found by Johnson (3) to cause water congestion in their leaf tissues and thus to predispose them to disease attack. This was demonstrated in relation to various rust and anthracnose diseases and also to Phytophthora blight of potatoes and tomatoes.

Exact knowledge of the effect of irrigation methods on Phytophthora blight on potatoes seems to be lacking. Cox and Large (1), in their recent survey of blight epidemics, confine themselves to the conclusions that (a) in Cyprus there is no evidence that the furrow irrigation practiced has any effect on blight, and (b) in Australia and New Zealand the increasing use of overhead irrigation may affect the incidence of blight attacks.

Both these conclusions are borne out by our experiment carried out under practically rainless conditions.

Surprisingly the presence or absence of dew had little effect on disease development. As overhead irrigation only slightly increased the number of dew hours per night its effect on dew seemed to be of little consequence in promoting disease development.

The level of relative humidity was raised by overhead irrigation only during the actual period of water application and subsequently for 2 to 3 hours. On plots irrigated once every 7 to 10 days this addition of high humidity periods may have assisted blight to develop more profusely than on plots irrigated less frequently, especially when the predisposing effect of direct leaf moistening is taken into account. However, it could then have been expected that low rates of overhead application, for 10 to 12 hours, would favor the disease more than 3 to 4 hours of irrigation at a more rapid rate; that this was not the case suggests that an additional factor is involved.

Such a factor could be the distribution of inoculum by splashing, as mentioned by Menzies (6). If so, rapid overhead irrigation with large droplets should certainly be more active than irrigation with small droplets. It may well be that this splash-distribution of inoculum plays an important role in development of blight under overhead but not under furrow irrigation. The lack of appreciable differences in blight development between plots under overhead irrigation at high or low rates could be speculated to be due to a cancelling-out effect, as follows: Under rapid irrigation inoculum is effectively splashed about, but high moisture periods are relatively short. Under slow irrigation the moisture periods are much more prolonged, but the inoculum is splashed about less energetically.

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PEAR DECLINE ON IKARIA ISLAND

A. G. Plakidas[1]

Apropos of a recent paper by T. A. Shalla, et al.[2], I wish to cite another case of a disease with symptoms very similar to those of the Italian and American pear decline that has decimated the pear trees on the island of Ikaria, Greece. This report should be of historical interest to students of pear decline since the incidence of the disease on Ikaria probably antedates both the moria in Italy and the pear decline in North America.

The island of Ikaria is an elongated mountain 21 miles long, 5 to 8 miles wide, 600 to 1040 meters in height, running in an east-west direction in the southeastern part of the Aegean Sea. Farming is practiced for the most part on terraced fields on the entire northern side of the mountain and on a more limited area on the southeast side. Pears are not grown in orchards. A thorny wild pear (Pyrus amygdaliformis) grows widely on the island, and the edible pear (a local variety known as "Stravoradi" (crooked-stem)) is grafted onto this wild tree in place wherever it happens to be in the field. No fertilization, no cultivation, no irrigation, no spraying is practiced; yet, before the onset of the decline disease, pears thrived and were apparently free from any fungus or bacterial diseases or insect pests. More fruit was produced than the island could absorb and some was exported to neighboring islands.

During the writer's boyhood days on the island (in the first decade of this century) it was accepted as a fact that pears would not grow in the southeastern side of the island. Tradition had it that pears did grow there in the past, but that a mysterious disease had killed them "some years ago" and all efforts to replace them were unsuccessful. Nobody knew the reason for this failure, but it was accepted as a well-known fact that pears would not do in the southeastern (Phanari) area of the island. On the northern side of the mountainous island, on the other hand, the pear trees were thriving and producing good crops during this period. At the time of the writer's emigration (1912) there were several thousand (exact figures not available) pear trees growing on the northern side of the island.

When the writer visited the island in 1954, he was amazed to find hardly any pear trees in this area, and the few scattered survivors were moribund. The symptoms of these declining trees were very similar to those described for moria in Italy and pear decline in North America, small pale green leaves turning red in late summer, arrested growth, die-back. No examination of roots was made.

The wild pear is not affected until it is grafted. I was informed that when a graft is made, the scion makes a seemingly normal growth for the first 2 to 3 years, then it begins to show symptoms of decline and eventually both the scion and the stock will die. This bears a striking similarity to the tristeza disease of citrus.

The following discussion is admittedly speculative, but the facts of the case would seem to support the speculation. It is theorized that the cause of the disease is probably a virus that was introduced into the southeast area of the island sometime around the middle of the 19th century. The inhabitants of this part of Ikaria were seafaring people, owners of large sailing ships that sailed into every Mediterranean and Black Sea port. It is probable that infected material (young pear trees of a variety that caught a captain's fancy?) was introduced into the southeastern area of the island from some foreign port. It must be further assumed that an insect capable of transmitting the virus was present on the island. It took the disease (infected plant material, insect vector?) many years to cross the mountain over to the northern side of the island. The best information I could get was that pear trees there began to fail around 1925 or 1930.

Pear trees are still growing and thriving in a small area at the northwestern end of the island. This area is separated from the rest of the island by a pine and oak forest and it is not unreasonable to assume that this forest has served as a barrier to keep out the hypothetical vector of the hypothetical virus.

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[1] Professor Emeritus, Louisiana State University, Baton Rouge.

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CONTROL OF FUSARIUM WILT OF TOMATO WITH VORLEX

P. A. Young[1]

Fusarium wilt of tomato (caused by Fusarium oxysporum f. lycopersici) formerly was very destruction in east Texas but is commercially controlled by crop rotation and use of immune tomato varieties such as Homestead. However, chemical control is desirable for small areas of very valuable land where rotation is impractical. Six cement-bordered plots 4 x 12 feet contained Eustis (Norfolk) loamy fine sand that was abundantly, naturally infested with the wilt fungus. Each plot was divided crosswise in half with sheet metal set 12 inches deep in the soil; alternating plots were reserved for untreated checks. The other six plots were treated with methyl isothiocyanate having chlorinated C_3 hydrocarbons (Vorlex[2]) at the rate of 58 gallons per acre. The Vorlex was injected into the soil in holes 10 inches apart when the soil temperature was at 60° F on February 14, 1961. Eight tomato plants with moderate polygenic resistance to wilt (Rutgers type) were set in each plot on March 27.

By April 17, the plots treated with Vorlex showed excellent control of weeds, especially Panicum texanum and Digitaria sanguinalis, that were abundant in the check plots. For about 2 weeks starting about April 26, the tomato plants growing in the soil treated with Vorlex showed prominent epinastic curling of the leaves in contrast to the normal leaves of the plants in the check plots. Unless they wilted, the tomato plants grew and yielded well in the plots until the end of the experiment on August 13, 1961. Vorlex controlled 72% of the wilt until the end of the marketing season (Table 1).

Table 1. Effect of Vorlex soil treatment on tomato wilt. 1961

Chemical treatment	% of tomato plants with Fusarium wilt on dates:							
	June 4	June 24	July 9			July 30		
	Wilted but alive		Normal	With wilt but alive	Killed by wilt	Normal	With wilt but alive	Killed by wilt
Vorlex	8	12	78	22	0	72	24[a]	4
None (checks)	27	82	4	24	72	4	2	94

[a] These plants remained alive during 6 weeks or longer after they showed symptoms of Fusarium wilt.

By July 30, 28% of the tomato plants showed wilt symptoms in the plots treated with Vorlex. It was especially interesting to see that these plants died very slowly; many of them lived during 6 weeks or longer with prominent symptoms of Fusarium wilt in one or two stems per plant. This delayed dying of the diseased plants indicated possible systemic effect of the Vorlex by increasing wilt resistance. Possibly this was associated with the earlier epinasty of the tomato leaves. In the check plots, the tomato plants died with wilt within a week or two as usual.

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[2] Vorlex supplied by the Morton Chemical Co., Woodstock, Illinois.

A SURVEY OF CARNATION DISEASES IN SOUTH SWEDEN

Gosta I. Nilsson[1]

Summary

To determine the cause of the carnation wilt in Swedish carnation greenhouses, a survey was carried out in southern Sweden during the summer of 1960. Sixty plants in an early stage of wilting were examined for presence of pathogens by plating 3-4 mm stem discs on PDA (potato-dextrose agar) in Petri dishes. Phialophora cinerescens (Wollenweber) van Beyma was isolated in 41 cases and was found to be the most serious pathogen of carnations in the investigated area of Sweden. Pectobacterium parthenii var. dianthicola Hellmers and Fusarium roseum Lk. emend. Snyd. & Hans. were each isolated in four cases. Fusarium oxysporum Schlecht. f. dianthi (Prill. & Del.) Snyd. & Hans. was found in one greenhouse, and was isolated from a carnation plant that was sent to the investigator by a carnation grower in Finland.

INTRODUCTION

Carnations are among the most important cut flowers in Sweden. Since the summer is relatively short and cool, quality cut flowers are produced only in greenhouses.

In 1957 the author was engaged in the inspection of carnation cuttings imported to Sweden or produced in Sweden either for export to other countries or for use by Swedish carnation growers. As in most other carnation-growing areas, diseases are of great importance, and for years Swedish carnation growers have been concerned with what has been called "carnation wilt." Hellmers reported in 1958 (1) and in 1960 (2) that during his investigation of carnation diseases in Denmark he also found the carnation wilt organisms Phialophora cinerescens and Fusarium oxysporum f. dianthi in Sweden, but it was evident that there was need for a more extensive investigation of carnation diseases in Sweden.

Since no funds were available the author carried out this investigation mainly with his own money and in his spare time, which of course limited the survey considerably[2].

MATERIAL AND METHODS

During June, July and August of 1960 the author visited 15 carnation growers in southern Sweden and examined one diseased carnation specimen from a carnation grower in Finland. Among the growers, two were producing cuttings for sale to other growers in Sweden and abroad. In spite of a thorough inspection of the mother plants and several hundred test tubes from the culture indexing laboratory of one of the carnation nurseries, no vascular pathogens could be found in the cuttings or in the mother plants.

When the carnation ranges and nurseries were visited, four to six 15-cm long pieces from stems and branches of wilting carnations were cut, labelled (grower, variety, and date) and put into plastic bags after first being wrapped in pieces of paper. The specimens were cut from the plants at a height where internal discoloration could be observed, in most cases about 20 to 30 cm above soil level. If no discoloration could be seen, samples were taken at the base of the plant and from different heights up to about 60 cm above soil level. Usually the samples were taken only from one plant in each bench or from one plant at the periphery of each locus of wilting plants.

In the laboratory, the ends of the specimens were cut off so that pieces 6 to 8 cm remained. These pieces were surface sterilized by rubbing them with a cloth dipped in 70% alcohol + 0.1% 8-hydroxy-quinoline sulfate. The pieces were then flamed and from the middle of each piece 6 to 8 discs, 3-4 mm thick, were cut off with a scalpel. The discs were placed on PDA in Petri dishes, which were left on the table in the laboratory (22° ± 4°C) for 10 days. The dishes were then examined and any organisms present were identified.

Although the aim of the investigation was mainly to find the cause of the wilt diseases, any other diseases encountered during the survey were noted.

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[2] The author wishes to express his thanks to Professor Ingvar Granhall, Chief of the Swedish Plant Protection Institute, for his interest in this work.

SURVEY RESULTS

Varieties showing wilt and the wilt pathogens isolated are listed in Table 1. In addition to the pathogens listed the following disease-causing fungi were found on leaves and stems of carnations: Alternaria dianthi Stevens & Hall (Fig. 1, F and G a), Alternaria dianthicola Neergaard (Fig. 1, G b), Heterosporium echinulatum (Berk.) Cke., Uromyces caryophyllinus (Schrank) Wint. Virus diseases of the streak, ringspot, vein mottle, and mosaic types were observed as well.

Table 1. Diseased carnation varieties examined in survey and wilt pathogens isolated.

Variety	Unknown cause	Phialophora cinerescens	Pectobacterium parthenii var. dianthicola	Fusarium oxysporum f. dianthi	Fusarium roseum
Cardinal Sim	1	1			
C. C. Mamie Sim		1			
C. C. Pink Sim/58		1			
C. C. Red Sim		3			
Creme Sim					1
Crowly Sim		2			
Flamingo Sim	1			1[a]	
Guldregn		2			1
Improved Pink Sim		1			
Laddie Sim	1	2			
Petterson's Pink		2			
Salmon Pink			1		
Saugus Pink		1			
Scania	2	8			1
Sidney Littlefield	3	4	1		
Solviks Sauges Pink		3			
White Sim		3			
William Sim		2			
Yellow Sim		1			
Yellow unknown variety		1			
Unknown varieties	1	3	2		1
Total	9	41	4	1	4

[a] Fusarium oxysporum f. dianthi was also isolated from a carnation plant of an unknown variety that was sent to the investigator from a grower in Finland.

PATHOGENICITY TESTS AND MORPHOLOGICAL STUDY OF THE PATHOGENS

Since there was no time or space for pathogenicity tests with all the isolated organisms, only Phialophora cinerescens, among the vascular organisms, was tested on carnations.

A 10- day-old culture of P. cinerescens from isolate C. C. Pink Sim/58-KP was used for further study. Twenty spores were measured and their average size determined as 4.8-2.4 μ (3.6 - 7.2 x 1.8 - 2.6 μ). The endogenic conidial formation from the branched conidiophores could be observed under the microscope (Fig. 1, E).

On July 8 two plants of the variety White Sim were inoculated with Phialophora cinerescens at the time of planting by placing pieces of a 10-day-old PDA culture close to the roots which had just been wounded by cutting off 1 cm of the root tips with a flamed scalpel. Two other plants were wounded in the same way but "inoculated" with sterile agar as a control. The plants were planted in 4-inch pots in Fruhsdorfer standard soil mixture and kept in the laboratory at about 22° ± 4°C. Once a week they received a complete fertilizer ("Substral").

One of the plants that had been inoculated with P. cinerescens showed the first symptoms of infection on August 25 in the form of reddening of the leaves. Two days later the other inoculated plant started to show the same type of red mottling of the leaves. The plant that first showed symptoms was cut into pieces on the same day and discs from the stem, 8 to 12 cm above soil level, were placed on PDA after surface sterilization. Three days later P. cinerescens could be identified growing from the discs. These discs showed browning of the vessels. The control plants were healthy and no pathogens could be isolated from the stems.

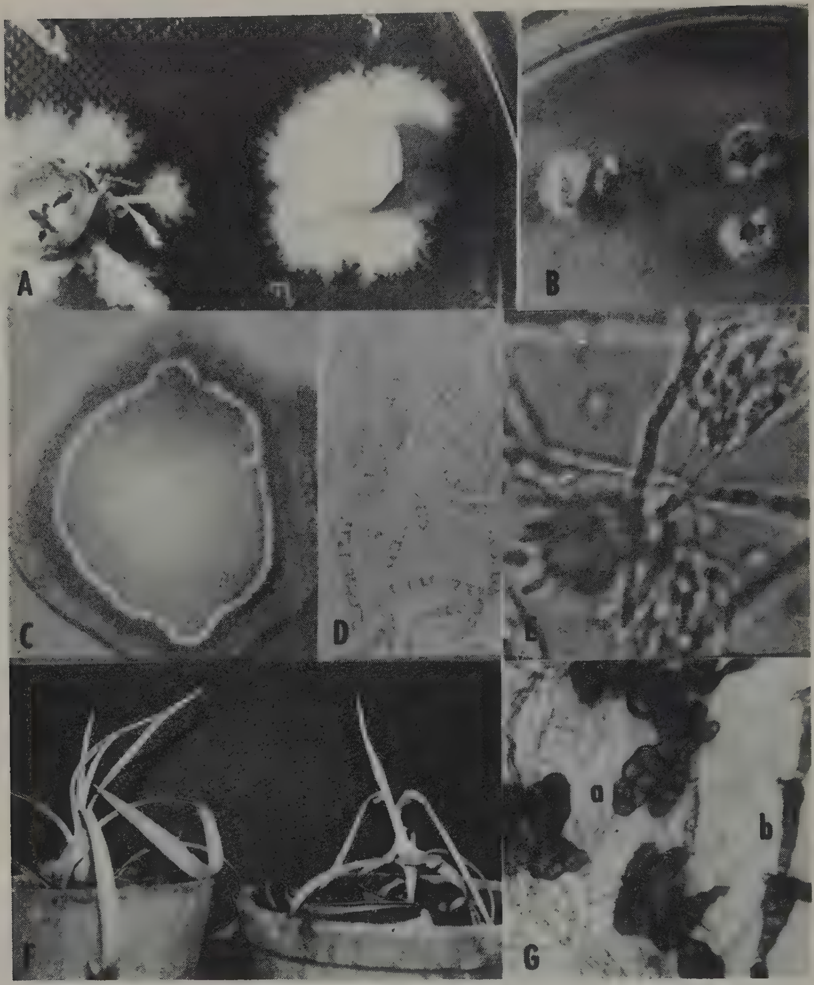

FIGURE 1. A -- Pectobacterium parthenii var. dianthicola growing from discs of Salmon Pink 10 days after plating on PDA (approx. X 1 1/2). B -- Phialophora cinerescens 70 hours after plating on PDA (approx. 3/4 natural size). C -- Stem section of a carnation plant infected with Fusarium oxysporum f. dianthi (approx. X 6). D -- Macroconidia of different age of Fusarium oxysporum f. dianthi (approx. X 300). E -- Branched conidiophores of P. cinerescens (approx. X 750). F. Right, carnation plant infected with Alternaria dianthi, 3 days after inoculation; left, control plant. G -- Two leaf-spot and branch-rot Alternaria-species in mixed culture for comparison of size and shape: a) Alternaria dianthi; b) Alternaria dianthicola (approx. X 169).

Since there has been some question whether Verticillium albo-atrum can cause wilt in carnations, two isolates of this fungus were tested for pathogenicity on the carnation variety Lilli-Ann. One of the isolates was obtained from the substation of the Swedish Plant Protection Institute in Akarp. The fungus had been isolated from the base of a wilted carnation plant. The other isolate of V. albo-atrum was isolated from a wilting tomato plant. Inoculation of wounded plants was done in the same way as described above for P. cinerescens. None of the isolates proved to be pathogenic to carnations under the above laboratory conditions. The fungus could not be isolated from any part of the plants when stem-pieces were plated on PDA more than 2 months after inoculation.

Pectobacterium parthenii var. dianthicola Hellmers was isolated from wilting carnation plants in two carnation greenhouse ranges. No pathogenicity test was carried out on carnations, but the bacterium was tested on sterile pieces of potatoes which were disintegrated in 10 to 24 hours at 28° to 30° C. On PDA the bacterium formed a type of colony that fit well with the description given by Hellmers (1). Especially characteristic were the lobated margins of the colonies (Fig. 1, A) and the soapy odor that developed from the culture on PDA. The bacterium was also found to be rodshaped and gram negative.

Fusarium oxysporum Schlecht. f. *dianthi* (Prill. & Del.) Snyd. & Hans. was identified by means of the descriptions given by Hellmers in his recent paper on carnation fusarioses (2) and by Wollenweber and Reinking (6). The disease caused by this fungus is usually called Fusarium-wilt, but Hellmers suggested the new name "white-vessel-fusariosis," referring to the white appearance of the vessels in infected plants (Fig. 1, C). The fungus formed abundant microconidia on PDA but very few macroconidia. When, however, stem pieces were kept in moist plastic bags the fungus grew through the cortex and formed abundant macroconidia on the stem surface (Fig. 1, D). From a wilted carnation plant sent from Finland, *Fusarium oxysporum* f. *dianthi* could be isolated from the stem vessels at the base of the plant and at different heights up to a point 10 cm below the flower. The white vessels appeared very distinct when the stem was cut transversely (Fig. 1, C). *Fusarium roseum* Snyd. & Hans. (*F. culmorum* (W. G. Sm.) Sacc. and *F. avenaceum* (Fr.) Sacc.) was isolated from the base of four wilting carnation plants, which had no vascular discoloration higher up in the stem but had brown roots and discoloration at the very base of the stem. The F. roseum-infected plants could also be recognized on the greyish green appearance of the foliage. In some cases *Fusarium roseum* was found to cause die-back of branches. Wounds from the breaking of cuttings and flowers were found to be the most common infection courts. During the investigation *F. roseum* was also isolated from newly rooted cuttings which had a brown zone of rotting tissue at the base. Of 50 diseased cuttings, 32 harbored the *avenaceum*-type, 9 the *culmorum*-type, 8 had both the *culmorum*- and the *avenaceum*-type, and one of the cuttings harbored a *Fusarium* species that was like the *culmorum*-type in growth although the macroconidia differed somewhat in shape.

Among the leaf-spot-causing fungi, *Alternaria dianthi* was tested for pathogenicity on young plants. The foliage of the plants was dipped in a spore suspension of the fungus before planting, and the plants kept under a plastic cover for 5 days in the laboratory. Within 3 days after inoculation the fungus caused the leaves to droop (Fig. 1, F) and on the fifth day the plants were completely wilted by the action of the pathogen. This fungus causes considerable damage in carnation greenhouses when the plant foliage is kept too moist.

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ATTRITION OF A SAGUARO FOREST[1]

Stanley M. Alcorn and Curtis May[2]

Summary

Between January 1942 and January 1961 bacterial necrosis destroyed 29.3% of a stand of saguaro near Tucson, Arizona. Significantly less mortality of plants 13 feet or less in height occurred than of those 13 to 19 feet in height. Evidence is presented that the size of a group does not increase the percentage mortality in the group, but that mortality is influenced by the proximity of saguaros to a dead plant. The numbers of saguaros surviving attrition by bacterial necrosis and wind follow a statistically significant linear decline. The continual reduction of the mature plant population and the lack of repopulation threaten the existence of the forest studied.

INTRODUCTION

Necrosis caused by the bacterium Erwinia carnegieana Standring has been recognized as a major disease of saguaro (Carnegiea gigantea) since the early 1940's (2, 5). This report presents the results of a study of saguaro mortality from the disease and other causes between 1942 and 1961 in plots in the Saguaro National Monument, Tucson, Arizona.

PROCEDURES AND RESULTS

In 1941-1942 six 10-acre plots were randomly selected from 64 plots comprising section 17 of the Saguaro National Monument. Maps showing the location of each saguaro were prepared and a number was assigned to each plant[3]. The age of a saguaro roughly relates to its height (3, 8). Further, a plant does not usually begin to flower until it is 8 to 10 feet in height or to develop branches until it is 16 to 22 feet in height. To determine whether these age-physiological changes affect mortality from bacterial necrosis, hereinafter referred to as BN, all saguaros were grouped arbitrarily into the following five height classes: I-0-6.9, II-7-12.9, III-13-18.9, IV-19-24.9, and V-25 feet and over. All plants were classified for size again in 1960. In 1941, 48 obviously diseased or dead saguaros were removed as part of an attempt to control the disease by eradication. Twenty-two additional plants were removed between 1942 and 1946. Beginning in 1943 the plots were surveyed annually (except in 1949 and 1955), usually in January or February, for mortality during the preceding calendar year. Mortality was recorded as the result of bacterial necrosis (BN), wind, eradication, and "other" (destruction by rodents, vandalism, and so forth).

Data obtained on all plants from the time the plots were established through the survey completed in January 1961 are summarized in Table 1. For computation purposes 22 plants (21 in class I and 1 in class IV), added to the survey since 1943, have been considered as having been present at the time of plot establishment, that is January 1942.

Initially for each 10 acres the average number of plants of known size was 244, with a range of 135-359. Five percent of these were estimated to be less than 7 feet and 47% more than 25 feet tall. In 1961 the population averaged 154 plants per 10 acres, of which 6% were estimated to be less than 7 feet and 49% more than 25 feet tall.

Apart from the eradicated plants, BN alone killed 432 saguaros, or 29.3% of the original population, since the start of the study. Windfall destroyed an additional 2.4%. When all eradicated plants and those destroyed by "other" means are omitted from the original population of saguaros, BN and wind caused a constant decrease in the remaining population (Fig. 1). The regression equation for this decline is $y = 1,144.59-25.65(x-10)$, with y, the ordinate, equal to plant population, and x, the abscissa, equal to years since 1942 (1942 + x years). The

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[1] Contribution from the Arizona Agricultural Experiment Station, Technical Paper No. 695. Appreciation is expressed to the National Park Service for permission to establish the study plots at the Saguaro National Monument, and to J. R. Hastings and Dr. Henry Tucker, University of Arizona, for reviewing the statistical analyses presented.

[2] Pathologists, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Tucson, Arizona, and Beltsville, Maryland, respectively.

[3] The plots were established and observed until 1954 by personnel of the Division of Forest Pathology, which formerly was responsible for the research.

Table 1. Mortality of saguaro in a forest near Tucson, Arizona, from January 1942 through January 1961.

Height classes	Plants in 1942 no.	Plants in 1942 %	Plants killed by: Bacterial necrosis no.	Bacterial necrosis % of class	Wind no.	Wind % of class	Eradication no.	Eradication % of class	Other means[a] no.	Other means[a] % of class	Total destroyed no.	Total destroyed % of class	Plants alive January 1961 no.	Plants alive January 1961 % of original grand total
I	78[b]	5.3	10	12.8	0	0	1	1.3	11	14.1	22	28.2	56	3.8
II	111	7.5	20	18.0	3	2.7	1	0.9	0	0	24	21.6	87	5.9
III	247	16.7	94	38.0	6	2.4	13	5.3	0	0	113	45.7	134	9.1
IV	340	23.1	113	33.2	13	3.8	20	5.9	0	0	146	42.9	194	13.2
V	692	46.9	195	28.1	14	2.0	28	4.0	0	0	237	34.2	455	30.8
Unknown	7	0.5	0	0	0	0	7	100.0	0	0	7	0.5	0	0
Total	1475	100	432	29.3	36	2.4	70	4.7	11	0.7	549	37.2	926	62.8

[a] Destruction by rodents, vandalism, and so forth.

[b] Values commonly bracketed do not significantly vary from each other at the 95% level of confidence. Bracketed values connected by dashes belong to a statistically related group.

correlation coefficient, r, equals -.9966 and the standard error for the regression coefficient, b = ± .5519. Assuming no changes in the rate of decline in the future, this plant population will theoretically (at the 95% level of confidence) cease to exist between 1995 and 1998.

Whether or not plants destroyed by wind, or removed for sanitary disposal, and "other" reasons were omitted from the original total population, analysis of variance and the Duncan multiple range test (4) showed that of the remaining class populations, saguaro mortality from BN was, percentage-wise, greatest in the class III group. This percentage was significantly greater, at the 95% level, than the mortality in classes I and II, but not significantly different from the mortality in classes IV and V. At the 99.0% level of confidence class V had a significantly greater percentage of the total population than in the other classes. Also, death from BN was greatest in class V (Table 1). No significant differences in mortality occurred between stands of saguaros in the six field plots.

The data were analyzed to determine whether proximity of plants affected mortality from BN. Plants destroyed by wind, eradication, or "other" reasons were considered healthy in these computations. Saguaros were arbitrarily considered as being in a group when a given plant was within 6 feet of another. On this basis there were 815 isolated plants of which 231 (28.3%) were killed by BN. In contrast, 126 groups (343 plants) had no mortality, 49 groups (133 plants) contained 1 plant each (36.8% of all plants in these groups) killed by BN, and 63 groups (184 plants) contained 2 or more plants (82.6% of all plants in these groups) killed by BN. In the latter grouping, 88% of all deaths from BN occurred within 6 years of initial deaths. Groups without deaths from BN were significantly greater than those with deaths. At the 97.5% level the groups with 2 + BN-killed plants had a greater percentage loss than did those groups containing only 1 BN-killed plant. In the three categories of plant groupings, each group contained essentially the same average number of plants. This similarity strongly suggests that the size of a group does not influence mortality, but that the proximity of the plants in a group to a BN-killed plant does.

DISCUSSION

Most saguaros begin to bloom when approximately 8 to 10 feet in height (that is, while in our class II). All plants bloom profusely by the time they are 13 feet in height. Thus, most of the present population in the plots is capable of producing seed. Although the number of plants was reduced by approximately 10 per acre between 1942 and 1961, recent information on the pollination requirements of the saguaro (6) suggests that this population (about 15 plants per acre) should produce sufficient viable seeds (estimated to be 2,000,000 per year) to maintain, if not increase, this population.

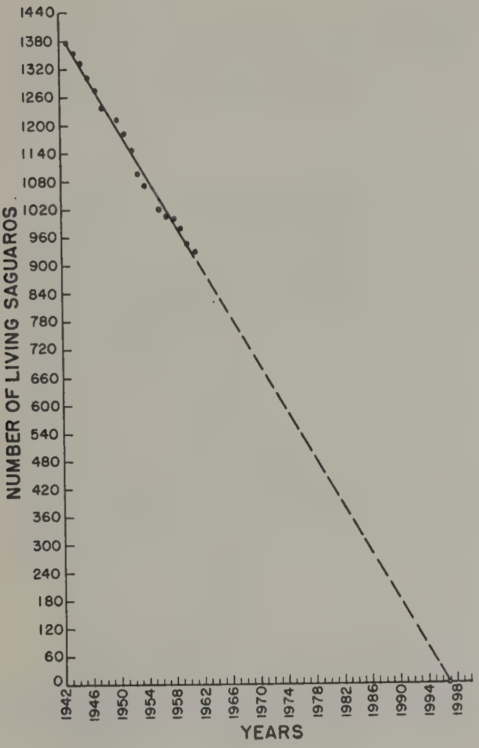

FIGURE 1. Saguaro mortality from bacterial necrosis and wind in a forest near Tucson, Arizona, between 1942 and 1961, and projected deaths through 1998.

In a stable or expanding population the ratio of juvenile to adult plants should be comparatively large (7, p. 114). Since the height of a saguaro can roughly be related to its age, that is, the taller the plant the older it is (3, 8), the sparsity of juvenile plants indicates that this saguaro forest is failing to maintain an adequate rate of natural repopulation. Thus, with the present estimated seed production, the conditions detrimentally affecting repopulation must primarily relate to seed-dispersal mechanisms, seed-germination requirements (1), and seedling establishment and survival. The continual attrition of this forest by BN and wind is, however, eroding the base for repopulation, the flowering plant. The data confirm the observations of Boyle (2), and indicate that once BN is established in a cluster of saguaros the remaining plants of the group are more apt to be killed than isolated plants. Thus, in any program of reforestation transplants should be well spaced.

The significant increase in mortality from BN between plants in group II, which are largely beginning to flower, and in group III, which have reached flowering size, is of interest. The increase in mortality of plants from BN after they are fully capable of blossoming suggests some relation to physiological changes related to flowering that might increase the susceptibility of a plant to BN. Flowering plants may also be more attractive to vectors (2) of the causal organism.

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APPLE SCAB II. EFFECT OF SERIAL APPLICATIONS OF FUNGICIDES ON LEAF LESIONS ON PREVIOUSLY UNSPRAYED TREES[1]

J. W. Heuberger and R. K. Jones[2]

cf. 41, 316

Summary

One field experiment was conducted in 1961, on apple trees previously unsprayed that season, to determine the effect of serial application of certain fungicides on apple scab leaf lesions. Data were obtained on (a) spore germination and (b) on late-season terminal leaf scab development. The data showed that 65% dodine (Cyprex) at 1/2-100[3] was as effective as at 1-100 in serial applications for suppression of spore germination for the time period (June 16-July 19) of the test; 50% captan (Captan 50W) 2-100; folpet (Phaltan 50W) 2-100, and captan + 75% zineb (Dithane Z-78) 1-1-100 were not so effective as dodine. The data on terminal scab development showed dodine to be much more effective in control than the other fungicides.

In a commercial Rome Beauty block not sprayed until the full bloom stage, leaves with scab lesions were tagged for weekly observation on development and for use in spore germination tests. The block received five applications of dodine (May 4-June 10) followed by captan + zineb for the remainder of the season. Data obtained showed: (a) after five applications of dodine, all parts of the sprayed lesions were purplish-red in color, no growth of the organism was visible macroscopically, the few conidia obtained by scraping did not germinate, and all heavily infected leaves had turned yellow and fallen; (b) on October 28 (36 days after the last application of captan + zineb), growth of an organism was visible on a few of the old lesions -- microscopic examination of the leaves after clearing in lactic acid showed the organism to be an *Alternaria* sp., not *Venturia inaequalis*; (c) late terminal infection was practically non-existent in the block.

INTRODUCTION

The tests reported here are a continuation of the long-range study, begun in 1960, on the effect of fungicides on previously unsprayed apple scab leaf lesions. In the first report of this study, Albert and Heuberger (1) reviewed the pertinent literature and presented data obtained in three experiments in 1960 on the effect of one application of fungicides on leaf lesions. These data showed that dodine appeared to have the most pronounced effect of all the fungicides tested on suppression of lesion development and spore germination. None of the fungicides appeared to have any effect on the organism under the cuticle of the leaf.

The purpose of the work reported here was to determine the effect of serial application of certain fungicides on suppression of spore germination and the subsequent development of late-season terminal scab. One experiment and an orchard demonstration were conducted under Delaware conditions.

MATERIALS AND METHODS

The fungicides used were 65% dodine (Cyprex), captan (Captan 50W), folpet (Phaltan 50W), and captan + 75% zineb (Dithane Z-78). The spore germination tests were made using the leaf disc-depression slide technique described by Powell (2).

Research Test: This test was conducted in a block of the Rome Beauty variety in which 16 trees had not been sprayed with a fungicide prior to the start of the test on June 16 (the approximate end of the primary infection period). Scab infection was as high as the senior writer had ever seen it. Leaves with from 1 to 3 lesions were tagged just before the first application of the fungicides. The fungicides, concentrations, and number of applications used were: dodine

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[The investigations reported in this article and the following one were planned jointly by the authors prior to initiation of the work. In such a study, a certain amount of duplication is inevitable; however, since two different approaches to the problem were used, the papers complement one another.]

[2] Respectively, Professor and Research Fellow, Department of Plant Pathology, University of Delaware.

[3] Refers to pounds of formulated product per 100 gallons of water.

Table 1. Effect of serial application of fungicides on apple scab leaf lesions on previously unsprayed trees, Rome variety, Bridgeville, Delaware, 1961. Spore Germination Studies (Conidia) and Terminal Scab.

Material and concentration	Number[a] of applications	Percentage germination at various day intervals after[b]						Terminal scab[c]		
		1 Application:	6	13	20	27	33	Severity of infection		
		2 Applications:	-	-	8	15	21			
		3 Applications:	-	-	-		7	Aug. 30	Oct. 6	Nov. 2[d]
Untreated	None		85	97	87	71	84	3.1	3.9	4.0
Dodine (1-100)	1		0	0	0	0	2	1.5	2.2	2.4
	2		-	-	0	0	0	0.2	0.7	0.7
Dodine (1/2-100)	1		0	0	0	6	40	1.3	1.8	2.2
	2		-	-	0	0	0	0.7	0.9	1.2
	3		-	-	-	-	0	0.1	0.4	0.4
Captan (2-100)	1		0	80	99	52	76	2.2	3.0	3.2
	2		-	-	0	6	70	1.0	1.7	1.8
	3		-	-	-	-	0	1.2	1.8	1.8
Folpet (2-100)	1		0	67	18	57	90	1.9	2.1	2.4
	2		-	-	0	0	61	1.7	2.0	2.0
	3		-	-	-	-	0	1.5	1.8	1.8
Captan + zineb (1-1)	1		0	84	66	62	86	1.8	2.6	2.8
	2		-	-	0	56	24	1.2	2.0	2.1

[a] Time of applications: first, June 16; second, June 28; third, July 12.

[b] Percentage spore germination on the checks 40 days after start of test had dropped to 49; also, few spores present. Test discontinued.

[c] Severity Index for terminal scab: 0, no spots; 1, 1 spot per leaf; 2, 2-5 spots per leaf; 3, 6-10 spots per leaf; 4, over 10 spots per leaf. (One hundred terminals scored per treatment).

[d] 139 days after one application, 127 days after two applications, and 113 days after three applications.

(1-100), 1 and 2 applications; dodine (1/2-100), 1, 2, and 3 applications; captan (2-100), 1, 2 and 3 applications; folpet (2-100), 1, 2, and 3 applications; and captan + zineb (1-1-100), 1 and 2 applications. One tree only was used for each material, concentration, and number of applications. On June 16, all of the trees were sprayed with a hand gun, using a John Bean orchard sprayer operating at 450 psi pressure. On June 28, those trees receiving two applications were sprayed; and, on July 12, those trees receiving three applications were sprayed. Trees were sprayed to the run-off point at each application. Three trees were left as unsprayed checks. At stipulated times after spraying, five tagged leaves from each of the test trees were brought to the laboratory, spore suspensions made, and the percentage germination determined. This was continued until 33 days (July 19) after the first spray application. After this date, spore germination tests were discontinued as the spore load on the lesions had become very low and germination of spores from unsprayed lesions had dropped to less than 50%. Complete data are presented in Table 1.

The trees were observed weekly for the general appearance of the lesions and for terminal scab infection. Late terminal scab infection became evident on August 17 and counts of severity were made on August 30, October 6, and November 2. One hundred terminals were scored per treatment per tree. The data are presented in Table 1.

Orchard Demonstration: This was conducted in a commercial Rome Beauty block not sprayed until the full-bloom stage. Scab leaf infection was conspicuous. Leaves with lesions were tagged for future observation and spore germination tests. Dodine was then sprayed, using a Speed Sprayer, as follows: full bloom, May 4, 1/2-100; petal fall, May 9, 3/4-100; first cover, May 19, 1/2-100; second cover, May 29, 1/2-100; third cover, June 10, 1/2-100. Thereafter, the block was sprayed with captan + zineb (1-1-100), the last application being made on September 22. Insecticides used were Guthion, lead arsenate, and Kelthane.

The block was examined at weekly intervals from the petal-fall period until the end of the season to determine the effect of dodine on the leaf lesions present when spraying began. At each examination, lesions on the tagged leaves were examined with an 18X hand lens. Also, leaves were taken to the laboratory and spore germination tests made until 32 days after the last dodine application. After this date, spore germination tests were discontinued owing to the small number of spores present on the sprayed lesions. Leaves collected at the last examination (October 28) were brought to the laboratory, cleared in lactic acid, mounted in lactophenol, and then examined microscopically for the presence of organisms.

RESULTS

Research Test: After the second fungicide application, lesions on the tree receiving dodine (1-100) were beginning to turn a purplish-red color and the visible amount of the fungus was being reduced. This occurred also on the tree sprayed with dodine (1/2-100) after three applications. The same phenomenon occurred, but to a much lesser extent, on trees sprayed three times with the other fungicides.

The data in Table 1 on the effect of serial application of fungicides on suppression of spore germination show the following:

a. Under conditions of one application, dodine (1-100) suppressed germination for at least 27 days, dodine (1/2-100) for at least 20 days, captan (2-100), folpet (2-100), and captan + zineb (1-1-100) for at least 6 days.

b. Under conditions of two applications, dodine (1-100; 1/2-100) suppressed germination for at least 21 days, folpet (2-100) for 15 days, and captan (2-100) and captan + zineb (1-1-100) for 8 days. The time limits for the dodine concentrations were not reached before the test had to be discontinued.

c. The data on three applications are meaningless so far as dodine, captan, and folpet are concerned, as the effect could not be determined past 7 days before the test had to be discontinued.

The data in Table 1 on terminal scab development show that all the fungicides, even at one application, had a marked effect. As the number of applications increased, the effect increased. Three applications (June 16, June 28, July 12) of dodine (1/2-100) had reduced the severity of terminal scab on November 2 to 0.4 as compared with 4.0 (maximum) on the unsprayed.

Orchard Demonstration: Weekly examination of the leaves containing lesions when spraying with dodine began showed the following:

a. Spore germination tests, carried out weekly until 32 days after the last dodine application, showed that none of the few spores present germinated.

b. After two applications, the lesions showed purplish-red colored spots and the organism was beginning to disappear. Also, dodine apparently had acted on incipient (invisible) lesions since purplish-red flecking appeared on tagged leaves in areas where no visible lesion had been present earlier. After five applications, no new lesions were present, all parts of the sprayed lesions were purplish-red in color, few visible signs of the organism were found on the surface, and all heavily infected leaves had turned yellow and fallen.

c. On October 28 (36 days after the last application of captan + zineb on September 22, and 140 days after the last application of dodine on June 10), 25 tagged leaves were examined. Of the 47 lesions present, 8 showed slight signs of growth of an organism on the surface. These were cleared in lactic acid and mounted in lacto-phenol. Microscopic examination showed that the organism present was an *Alternaria* sp., not *Venturia inaequalis*.

d. Terminal scab infection was difficult to find on October 28.

DISCUSSION

It was unfortunate in the Research Test that the germination of spores on unsprayed lesions dropped to almost zero before the effect of serial applications of the fungicides could be determined fully. The last spore germination test reported (33 days after the first spray application) was made on July 19. In the next test, July 26, the germination had dropped to 49% and, in two subsequent tests 7 days apart, the germination had dropped to almost zero. In this connection, it is interesting to note that in the second Winchester, Virginia test conducted by Albert and Heuberger (1) in 1960 the percentage germination of spores from unsprayed lesions was 17, 43, 37, and 31 on July 20, 27, August 3, and 10, respectively. Apparently, climatic conditions in Delaware in 1961 and at Winchester, Virginia in 1960 were not conducive to spore germination in midsummer. However, in spite of the above, the data obtained on spore germination indicate that serial application of dodine (1/2-100) should suppress germination and, consequently, prevent terminal scab infection. Much more data are needed before a definite statement can be made.

The information obtained in the Orchard Demonstration on the effect of serial application of dodine indicates that five applications destroy the scab organism on the leaf surface. This confirms the observations of Weaver (4). However, much more information is needed, particularly under controlled conditions, before a definite statement can be made.

All the information at hand to date (1, 2, 3, 4, 5)[4] shows that dodine is superior to other fungicides in regular use in apple spray schedules for scab control. Dodine, however, does not have so wide a spectrum of apple disease control as some other fungicides. Its use in a regular schedule may be restricted to the primary scab infection period (usually from the delayed dormant application through the second cover application), particularly so in areas where sooty blotch, fly speck, and various fruit rots are an annual problem, as it is not so effective as some other fungicides in control of these diseases. Further, its use with lead arsenate is questionable in some areas from the standpoint of incompatibility, and lime cannot be used with dodine as a safener for lead arsenate. As a special spray under conditions of established scab infection, dodine is the first-choice material.

Evidence is beginning to accumulate that dodine may eradicate Venturia inaequalis from the leaf surface. If this is proved true by further research, then a material is at hand that should prevent late-season terminal scab infection that carries the organism overwinter to provide the source of primary infection in the spring.

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[4] Also, personal communication from Dr. F. H. Lewis, Arendtsville, Pennsylvania, to the senior author, dated December 6, 1960.

EFFECT OF REPEATED APPLICATIONS OF DODINE AND OF CAPTAN ON APPLE SCAB FOLIAGE LESIONS[1]

J. J. Albert and F. H. Lewis[2]

Summary

Formulations of dodine and captan were compared on visibly established apple scab foliage lesions. Data were obtained on spore numbers, spore germination and increase in diameter of the lesions. The treatments sharply reduced spore numbers in some instances, and the reduction varied with the fungicide, the spray interval and the sampling date. Spore germination was affected most by 65% dodine, which gave complete inhibition at 1 pound in 100 gallons of water. At concentrations of 1/2 and 1/3 pound, the spray interval and the number of spray applications affected the results. Neither dodine nor captan prevented increase in diameter of the lesions (fungus growth within the leaf). Dodine tended to eradicate all visible fungus growth from the surface of the lesions. It was concluded that both reduced inoculum and reduced spore germination were important factors in the control of secondary apple scab infection.

INTRODUCTION

One of the difficulties in controlling Venturia inaequalis (Cke.) Wint., the fungus causing apple scab, has been the lack of an effective, reasonably safe and economical fungicide program for those cases where severe foliage infection in the spring precedes normal to heavy rainfall in the summer. Fungicides that normally are quite satisfactory fail under the twin handicaps of abundant inoculum and rainfall.

Recent work has indicated that a reasonable solution to this problem has been found. Results obtained with single applications of fungicides were reported by Albert and Heuberger (1) and Powell (2). Weaver (3) reported observations following repeated applications of fungicide. This paper reports data obtained in Pennsylvania in 1961 on the effects of repeated applications of dodine and captan on apple scab foliage lesions at concentrations and timing that are normal in eastern apple orchards.

MATERIALS AND METHODS

Two fungicides were compared: dodine (used as Cyprex 65% wettable powder), and captan (used as Captan 50% wettable powder).

The test began June 27 and ended September 19, 1961. It was conducted on previously unsprayed 4-year-old Stayman Winesap trees using a randomized block design with eight treatments replicated six times. Leaves bearing individual scab lesions were selected and tagged before the first spray was applied. About 20 leaves were tagged on each tree. The sprays were applied from the ground using a hand gun at 550 pounds' pressure. All trees were sprayed with DDT and Kelthane (1-1-100) on July 11 and with Sevin (1-100) on August 7. The number of times a particular fungicide was applied varied according to the treatment (Tables 2 and 3).

One leaf was selected from each tree at each scoring date, making a total of six leaves from each treatment. Three leaves were used for determining the number of spores present per square millimeter of leaf surface. Three leaves were used for determining spore germination.

The number of spores per unit area of lesion was determined as follows: A single lesion was cut from each of three leaves with a cork borer of the same diameter as the lesion. Each lesion was dipped repeatedly for 1 minute in 0.11 ml water in each cell of a standard double-celled depression slide. It was then scraped lightly in a third depression cell to aid in removing

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[The investigations reported in this article and the preceding one were planned jointly by the authors prior to initiation of the work. In such a study, a certain amount of duplication is inevitable; however, since two different approaches to the problem were used, the papers complement one another.]

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Table 1. [a]Rainfall data for June, July, August, and up to September 19, 1961, Biglerville, Pennsylvania.

Date	June	July	August	September
1	0.02	--	0.15	0.04
2	Trace	0.16	0.44	0.06
3	0.03	--	0.10	1.06
4	0.37	--	--	--
5	--	--	0.06	--
6	Trace	--	--	0.05
7	--	--	--	0.95
8	--	--	--	--
9	0.07	0.04	--	--
10	0.34	--	Trace	--
11	--	--	0.02	--
12	--	0.21	--	0.08
13	--	0.12	--	--
14	--	0.25	--	0.06
15	0.50	0.03	--	--
16	--	Trace	--	--
17	--	0.10	--	--
18	--	0.18	--	0.03
19	--	0.98	--	Trace
20	--	--	0.22	
21	0.03	--	0.06	
22	1.94	--	0.07	
23	--	Trace	0.47	
24	0.04	0.41	0.86	
25	0.03	--	0.17	
26	Trace	--	0.10	
27	0.07	--	--	
28	--	0.48	--	
29	--	0.14	--	
30	--	--	--	
31	--	--	--	
Total	3.44	3.10	2.72	2.33

[a] Daily and total rainfall expressed in inches.

the spores. Then, each lesion was observed under a stereomicroscope to make sure that all of the spores were removed. The spores were counted and the area of the lesion in millimeters divided into the number of spores. The average of three lesions was used to represent the number of spores per square millimeter of lesion surface.

Three lesions were used on each recording date to determine spore germination. Three depression cells, each containing 0.11 ml rain water, were used for each treatment. The lesions were cut from the leaves with a cork borer, inverted, and the spore-bearing surface placed on the water. The amount of time the lesions were left on the water varied with the number of spores present on each lesion. A total of 1500 spores (500 from each lesion) was used to determine the spore germination at each scoring date. The slides were put in individual moist chambers in a 20°C constant temperature chamber for 24 hours. Germination was determined at the end of that time. The average of three lesions equaled the spore germination on each recording date.

Previously tagged leaves bearing individual lesions were used in measuring the diameter of each lesion. Six lesions on each of six trees were used for a total of 36 lesions per treatment. Enough leaves had been tagged so in case a few were lost others could be substituted. After tagging, the lesions were measured at intervals.

RESULTS

The data on rainfall, treatment dates, spore numbers, spore germination, and increase in lesion size are given in Tables 1 through 4.

The period from June 27 to about July 20 was favorable for the growth of the fungus, and most of the differences between treatments occurred during that period.

The number of spores (conidia) on the check or untreated lesions varied violently between the various sampling dates. For example, a lesion of the size which normally might be expected to have mycelium and spores over the entire surface (about 10 mm in diameter) had about 28,000 conidia present on July 3 following a light rain on July 2. By July 6, the number had increased to about 95,000 spores per lesion with no rainfall between the two dates. The number decreased on July 10 and 14 following light rainfall, and was very high again on July 17 during a period when rainfall occurred daily but probably was inadequate to cause much spore removal. Low spore numbers on and after July 20 are attributed to increasing age of the lesions and conditions unfavorable for fungus growth.

The treatments sharply reduced spore numbers in some instances, and the reduction varied with the fungicide, the spray interval, and the sampling date. Captan at 2 pounds/100 gallons of spray reduced spore numbers by about three-quarters within 3 days after the first application, and this reduction was maintained fairly well during the following 2 weeks with little rainfall.

Table 2. Suppression of Venturia inaequalis conidia by repeated applications of fungicides. The data represent the number of spores present per square millimeter of lesion surface. Biglerville, Pennsylvania, 1961.

		Spray dates for each treatment are listed as follows:						
		6/27 7/7 7/17 7/27 8/7	6/27 7/11 7/25 8/8 8/22	6/27 7/11 7/25 8/8	6/27 7/18	6/27 7/7 7/17 7/27 8/7	6/27 7/11 7/25	6/27 7/11 7/25 8/8
Record Date	Unsprayed	Dodine 65W (lb/100 gal.)				Captan 50W (lb/100 gal.)		
		1/3 lb 10 days	1/3 lb 14 days	1/2 lb 14 days	1 lb 21 days	1 1/2 lb 10 days	1 1/2 lb 14 days	2 lb 14 days
June 30	1035	670	562	366	207	333	379	213
July 3	354	103	100	111	63	216	201	185
July 6	1209	312	317	263	73	431	417	403
July 10	743	202	436	109	63	228	725	259
July 14	446	69	57	41	28	243	238	149
July 17	1132	179	173	97	126	969	932	887
July 20	143	64	99	74	55	102	128	118
July 25	75	40	81	47	24	54	101	93
July 31	237	20	35	40	38	90	121	103
August 7	103	46	36	33	35	65	70	63

Table 3. Percentage germination of Venturia inaequalis conidia after 24 hours at 20° C, Biglerville, Pennsylvania, 1961.

		Spray dates for each treatment are listed as follows:						
		6/27 7/7 7/17 7/27 8/7	6/27 7/11 7/25 8/8 8/22	6/27 7/11 7/25 8/8	6/27 7/18	6/27 7/7 7/17 7/27 8/7	6/27 7/11 7/25	6/27 7/11 7/25 8/8
Spores Collected	Unsprayed	Dodine 65W (lb/100 gal.)				Captan 50W (lb/100 gal.)		
		1/3 lb 10 days	1/3 lb 14 days	1/2 lb 14 days	1 lb 21 days	1 1/2 lb 10 days	1 1/2 lb 14 days	2 lb 14 days
June 30	95	8	7	0	0	2	1	0
July 3	89	26	27	0	0	39	37	0
July 6	90	24	27	0	0	36	34	0
July 10	91	0	59	41	0	1	67	4
July 14	90	0	0	0	0	79	36	0
July 17	91	0	0	0	0	87	81	73
July 20	85	0	0	0	0	71	79	75
July 25	90	2	8	0	0	80	90	84
July 31	87	0	0	0	0	27	21	9
August 7	80	0	0	0	0	70	59	31

Table 4. Increase in diameter of visible apple scab foliage lesions subjected to repeated applications of fungicides, Biglerville, Pennsylvania, 1961.

Material and concentration	Av. diam. (mm) of 36 lesions, June 27	Av. diam. (mm) of lesions July 21	Aug. 28	Sept. 19	Av. increase diam. (mm) June 27--September 19
Check	5.23	6.48	7.54	8.38	3.51
Dodine 1/3-100 every 10 days	4.97	6.45	7.49	7.88	2.91
Dodine 1/3-100 every 14 days	4.87	6.25	7.78	8.38	3.51
Dodine 1/2-100 every 14 days	5.09	6.48	7.54	8.19	3.10
Dodine 1-100 every 21 days	5.21	5.56	6.35	6.90	1.69
Captan 1 1/2-100 every 10 days	5.25	6.33	7.85	8.28	3.03
Captan 1 1/2-100 every 14 days	4.90	5.85	6.82	7.37	2.47
Captan 2-100 every 14 days	5.21	6.38	7.61	8.07	2.86

However, following the second application on July 11, 0.61 inches of rain fell in 4 days and captan was relatively ineffective by the sixth day. Captan 1 1/2 pounds at 10-day intervals gave substantially the same results as captan 2 pounds at 14-day intervals. With captan at 1 1/2 pounds at 14-day intervals, there was clear evidence that the treatment had partially failed between the tenth and fourteenth day after the first treatment. Dodine was more effective than captan, especially during the period in mid-July when rain fell daily.

Dodine at 1 pound at a 3-week interval caused a very sharp reduction in spore numbers which was maintained throughout the remainder of the experiment. There was some decrease in effectiveness with decreased concentration of chemical, but lesions sprayed with 1/2 and 1/3 pound dodine at 10- and 14-day intervals had substantially fewer spores than the checks on all sampling dates up to July 20.

Dodine at 1 pound prevented spore germination on all sampling dates after the first application. At 1/2 pound, the treatment prevented spore germination for at least 10 days but had partially failed near the end of the 2-week interval following the first application. No germination was obtained after the second application. At 1/3 pound, spore germination was decreased but not stopped until after the second spray. Even after the second spray, some germination occurred on July 25 at the end of both 10- and 14-day spray intervals.

While captan caused a reduction in spore germination in most instances, the reduction was inadequate to provide control under the conditions of this test.

No treatment prevented increase in diameter of the lesions, although dodine at 1 pound had some effect.

There was a very marked effect of some dodine treatments, especially at 1 pound, on the appearance of the lesions. With visible lesions, the fungus partially or completely disappeared from the surface and the lesion showed varying degrees of brown, purple, and yellow colors. Where the spray apparently was applied after infection but before the lesion became visible, the fungus continued to grow within the leaf but did not appear on the surface. Some leaves were mottled in appearance, others developed small brown to purple spots unrecognizable as scab lesions (Figs. 1 and 2). Both the upper and under sides of the leaves were affected. The under sides of the leaves remained brown to purple and no signs of the fungus appeared, whereas scattered lesions developed on the upper surface of the leaves in late August.

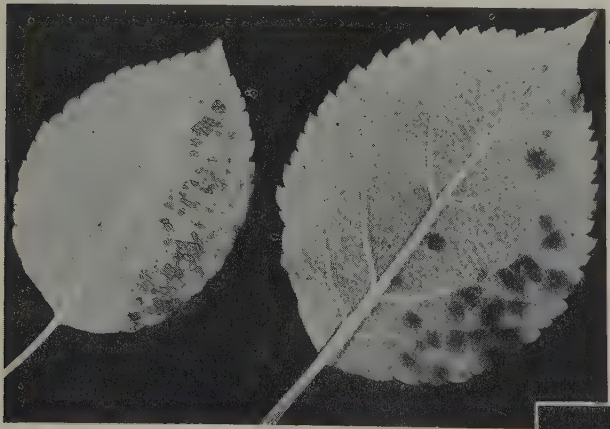

FIGURE 1. Effects of repeated applications of fungicides on apple scab lesions on the under side of the leaves. Left -- Leaf sprayed with dodine (1-100), two applications. Right -- Leaf sprayed with captan (2-100), four applications.

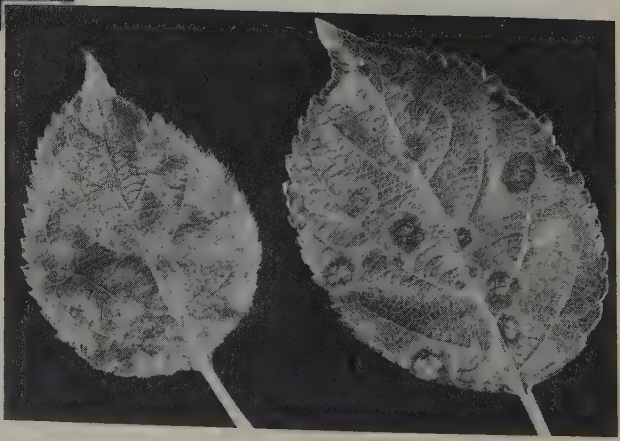

FIGURE 2. Effects of repeated applications of fungicides on apple scab lesions on the upper side of the leaves. Left -- Mottled effect on a leaf sprayed with dodine (1-100), two applications. Right -- Normal looking lesions on a leaf sprayed with captan (2-100), four applications.

DISCUSSION

An infection potential was determined on these trees before the test was started. Visible scab lesions were numerous, but not so severe as to cause extensive defoliation. Approximately 825 spores were present per square millimeter of scab lesion surface on each tree in the test block. About 90% of these spores germinated in rain water. The infection potential, therefore, would be classed as heavy although many cases have been observed where heavier rainfall and a greater number of leaf lesions created a more severe problem.

The data obtained in this and earlier work previously cited show that both reduced spore numbers and reduced spore germination may be important factors in the control of secondary apple scab infection. In the studies reported here, dodine was superior to captan, and seemed to owe some of its superiority to the fact that it was not greatly affected by the amount of rainfall encountered in 1961. The degree of effectiveness of dodine was such as to create some confidence that apple scab infection potential can be reduced to zero or near zero in spite of abundant inoculum on the trees. Also, the data support the idea that scab control can be obtained after the second or third post-bloom spray with spray intervals longer than 2 weeks.

The data on increase in lesion diameter support the previous conclusion by Albert and Heuberger (1) that neither dodine nor captan eradicates the scab fungus from within the leaf.

Further interpretation of the data needs certain supporting information, and must be limited to situations comparable to those in Pennsylvania. The normal interval between pesticide applications is now 2 weeks beginning about the second cover spray (third post-bloom). The population of Venturia inaequalis usually is low. Spray applications are reasonably thorough and well-timed in most instances. Total spray chemical costs per acre are high; dodine prices currently are about $2.45 per pound, and the chemical sometimes causes fruit russeting on some, possibly all, varieties when used at about 1 pound. Therefore, it is important to use the minimum concentration of chemical required to give a degree of control satisfactory for the purpose for which the fruit is intended.

Under these conditions, dodine at 1/3 pound concentrated and used at 1 pound/100 gallons in 3X concentrate at about 125 gallons/acre has been adequate for scab control and superior to competitive materials. However, this is a marginal concentration which may prove inadequate in some cases. One-half pound probably would be a better recommendation for an area covering several States.

Where severe infection in the spring precedes normal to heavy rainfall in the summer, our data suggest several possible courses of action where control is to be attempted. If the crop has been lost and defoliation is a serious threat, dodine at 1 pound at about 3-week intervals seems best. Where pedicel infection is not serious and the crop can be saved, dodine at about 2/3 pound followed by dodine at about 0.4 to 0.5 pound at intervals not exceeding 2 weeks should be adequate for the prevention of new infections. The control of other diseases probably would require the use of other, more effective fungicides late in the season.

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BACTERIAL BLIGHT OF COTTON IN ARKANSAS[1]

B. E. McCutchen and N. D. Fulton[2]

INTRODUCTION

Bacterial blight, caused by Xanthomonas malvacearum (E. F. Sm.) Dows., is one of the most damaging diseases of cotton. From 1956 through 1960 an average estimated yearly loss of 344, 829 bales of cotton was reported in the United States (2). For this same period in Arkansas an average yearly loss of 13, 527 bales was reported. Loss in yield due to bacterial blight makes this disease second in importance only to seedling diseases in this country.

During the past decade a number of varieties resistant to the disease were developed. Among them was Rex, an Arkansas release having Stoneville parentage. However, in 1954 Hunter and Blank (3) described a new strain of X. malvacearum that is pathogenic to cotton seedlings with Stoneville type resistance. This strain, later designated as race 2, has since been reported in several cotton-growing areas (1).

Since Rex is the only resistant variety grown to any extent in the State and because it is resistant only to race 1 the distribution and prevalence of the different races of the pathogen in the State are very important. The purpose of this investigation was to determine the distribution of races 1 and 2 of X. malvacearum in Arkansas.

MATERIALS AND METHODS

Thirty-seven diseased specimens were collected from 27 of the major cotton-growing counties of Arkansas.

Differential cotton varieties used were as follows: Z-106, susceptible to both race 1 and race 2; Stoneville 20, resistant to race 1 and susceptible to race 2; Mebane Bl, resistant to races 1 and 2; Rex, a commercial variety with Stoneville type resistance. The cotton plants were grown in the greenhouse in 2-gallon crocks of soil and inoculated when 6 to 8 weeks old.

Infected leaves from the collected specimens were ground up and thoroughly agitated in sterile distilled water. This bacterial suspension was decanted and used as inoculum. The inoculum was forcibly injected into the under surface of the leaves of the test plants by the use of a hypodermic syringe equipped with a piece of rubber tubing until a water-soaked area on the leaf was produced. Each isolate was tested on a total of five plants of each differential. Resistant or susceptible plant reactions were recorded 2 weeks after inoculation.

RESULTS AND CONCLUSIONS

Although seven isolates collected from four counties produced reactions for race 2, this study shows that race 1 of X. malvacearum is the more prevalent in Arkansas (Fig. 1). Some variation in virulence was noted among the isolates. However, these differences were thought to be quantitative as they were inconsistent when the isolates were re-tested. Results of this survey show that while race 2 is not present to any appreciable degree it is fairly evenly distributed throughout the cotton-growing region of this State. Since almost half of the cotton acreage is planted to Rex, selection pressure could possibly cause a build-up of race 2. Future surveys are planned to give an estimate of the distribution of the races of X. malvacearum in Arkansas.

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[1] Published with approval of the Director of the Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

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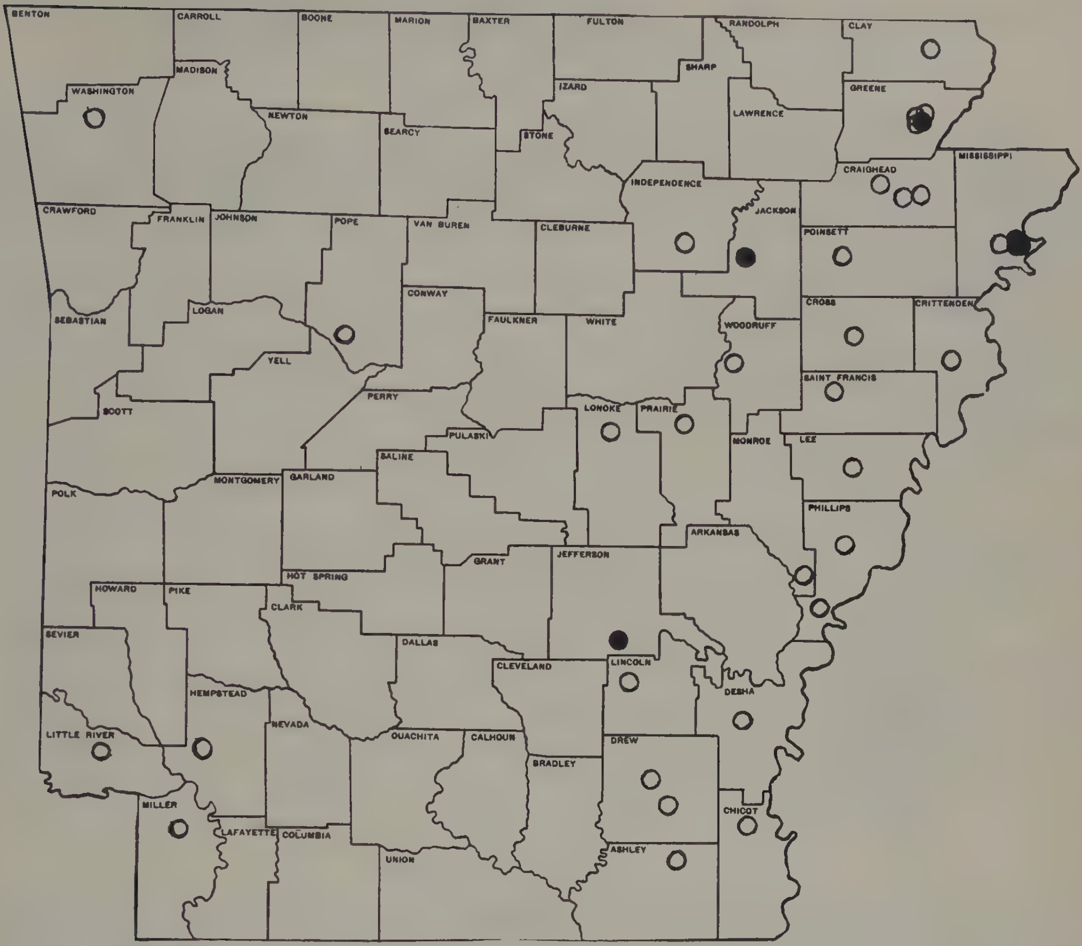
BENTON
CARROLL
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BAXTER
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MADISON
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SEARCY
STONE
LAWRENCE
MISSISSIPPI
CRAIGHEAD
INDEPENDENCE
JACKSON
CRAWFORD
FRANKLIN
JOHNSON
POPE
VAN BUREN
CLEBURNE
POINSETT
CONWAY
SEBASTIAN
LOGAN
FAULKNER
WHITE
WOODRUFF
CROSS
CRITTENDEN
YELL
SAINT FRANCIS
PERRY
SCOTT
LONOKE
PRAIRIE
PULASKI
MONROE
LEE
SALINE
MONTGOMERY
GARLAND
POLK
PHILLIPS
ARKANSAS
GRANT
JEFFERSON
HOT SPRING
HOWARD
PIKE
CLARK
SEVIER
DALLAS
CLEVELAND
LINCOLN
HEMPSTEAD
DESHA
NEVADA
LITTLE RIVER
OUACHITA
CALHOUN
DREW
BRADLEY
MILLER
CHICOT
LAFAYETTE
COLUMBIA
ASHLEY
UNION

FIGURE 1. LOCATION OF *XANTHOMONAS MALVACEARUM* RACES IN ARKANSAS ACCORDING TO 1961 SURVEY.

○ —RACE 1 *X. MALVACEARUM*

● —RACE 2 *X. MALVACEARUM*

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THE INHIBITORY EFFECT OF 2-CHLOROETHYLTRIMETHYLAMMONIUM CHLORIDE TREATMENT ON TOBACCO MOSAIC VIRUS (TMV) MULTIPLICATION

T. E. Rawlins[1]

The growth-regulating substance 2-chloroethyltrimethylammonium chloride, commonly called CCC[2], shows a relatively low toxicity to higher plants. A .5% solution of the compound did not cause detectable macroscopic injury to floating leaf discs in 5 days. After 6 days a faint chlorosis was produced. This report describes tests to determine the effect of this compound on TMV multiplication.

The heat-ultra method described by Schlegel and Rawlins (1, 2) was used in the experiments. The leaf discs were floated for 6 days. The check discs were floated on distilled water. The pH of a .5% solution of CCC is around 3.5. This was adjusted to about pH 7.0 by the addition of a small amount of 2 M phosphate buffer.

The results of the spectrophotometric assay of virus from floating leaf discs are shown in Table 1.

Table 1. Effect of CCC on TMV multiplication.

Treatment	Optical density 260 M	Average	% inhibition
Check #1	.423		
Check #2	.407	.436	
Check #3	.477		
.5% CCC #1	.043	.034	92%
.5% CCC #2	.025		
.1% CCC #1	.082	.084	81%
.1% CCC #2	.086		

The above data show that virus multiplication was much lower in the treated tissue than in the discs on distilled water.

It is hoped that other workers will explore further the possibility of using this compound in the chemotherapy of plant and animal viruses.

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[2] The CCC was kindly furnished by Dr. Robert E. Deems, American Cyanamid Company, Stamford Research Laboratories, 1937 West Main Street, Stamford, Connecticut.

NEW FINDINGS OF PLANT-PARASITIC NEMATODES IN PERU

J. N. Sasser, Oswaldo F. Vargas Gonzales, and Albert Martin

A survey of plant-parasitic nematodes in Peru was published by Krusberg and Hirschmann in 1958[1]. They identified 33 plant-parasitic species representing 14 genera. These included Criconemoides, Helicotylenchus, Hemicriconemoides, Hemicycliophora, Heterodera, Hoplolaimus, Meloidogyne, Paratylenchus, Pratylenchus, Rotylenchus, Trichodorus, Tylenchorhynchus, Tylenchulus, and Xiphinema.

Species of all the above genera were again isolated from soil and/or root samples collected during June, July, and August of 1961[2]. In addition, four plant-parasitic genera, not previously reported from Peru, were found. The purpose of this report is to record these new findings.

1) Ditylenchus dipsaci was observed on alfalfa in several fields in the Tarma Valley (11° 25' 40" south latitude, 75° 42' 50" west longitude) at an elevation of 9240 feet (3080 m). The nematode was causing considerable damage in two fields observed. A more thorough survey will be needed, however, to determine its distribution and importance in the production of alfalfa. It was not found in the coastal region or in the Selva (jungle) although several fields of alfalfa were examined in the coastal region and one planting in the Selva.

2) Rotylenchulus reniformis was found in the departments of Piura and Tumbes (5° 11' 51" to 3° 34' 16" south latitude and approximately 80° west longitude). Large numbers of this nematode, usually in almost pure populations, were isolated from soil collected from rhizospheres of cultivated cotton, orange, banana, cocoa, and tobacco plants. This apparently is the first report of this nematode from South America.

3) Radopholus similis was observed causing severe damage to banana in a plantation near Tumbes, Motupe (Lambayeque), Huarmey (Ancash), Pativilca (Lima), and Chincha (Ica).

4) Dolichodorus[3] occurred in soil collected from around the trunk of a mahogany tree growing on the Agricultural Experiment Station at Tingo Maria. Whether the mahogany tree was serving as a host was not determined. This is believed to be the first report of this genus from South America.

GENERAL OBSERVATIONS

Although several genera of plant-parasitic nematodes were found associated with various crops grown in the coastal region, the root-knot nematode Meloidogyne incognita, and the burrowing nematode Radopholus similis, appeared to be the most important economically, causing severe damage to cotton and banana respectively. Furthermore, there was considerable evidence of an interrelationship between these nematodes and certain fungus diseases. Root-rot caused by Thielaviopsis basicola and wilt, caused by Verticillium albo-atrum, were more severe in cotton fields infested with root-knot nematodes. In some cases, Radopholus similis was found in combination with the Panama disease, caused by Fusarium oxysporum var. cubense.

Although many genera of plant-parasitic nematodes were found associated with various crops in the Sierra, Tarma, Huasahuasi (Junin), Lago Titicaca (Puno) and Cuzco vicinity, Heterodera rostochiensis, causing severe losses to potato, appeared to be the most important economic species.

From a limited survey of the Selva (Tingo Maria and vicinity) the root-knot nematode, Meloidogyne exigua, on coffee appeared to be the most damaging.

Although the two surveys have been somewhat restricted, enough information has been accumulated to justify the conclusion that plant-pathogenic nematodes are limiting factors in the production of several important crops in Peru; these include cotton, potato, coffee, banana, and alfalfa. Research programs leading to the control of nematodes attacking these crops could be very rewarding.

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[1] Krusberg, L. R. and Hedwig Hirschmann. 1958. A survey of plant parasitic nematodes in Peru. Plant Disease Reptr. 42: 599-608.

[2] The senior author visited Peru during June, July, and August of 1961 under the technical aid contract which North Carolina State College operates for the Agency for International Development, Washington, D. C., working with SIPA (Servicio de Investigacion y. Promocion Agraria) Lima, Peru.

[3] An undescribed species.

A DISEASE OF SNOW-IN-SUMMER, CYPRESS EUPHORBIA, AND CORSICAN SANDWORT CAUSED BY A SPECIES OF RHIZOCTONIA

Lester P. Nichols[1]

In August 1961, a severe blighting of the foliage of snow-in-summer (Cerastium tomentosum) was observed in a demonstration of ground covers near Boston, Massachusetts. Thick wefts of hyphae of a species of Rhizoctonia were found growing over the surface of the irregular patches of blackened, blighted plants.

At the same time in a nearby planting of cypress euphorbia (Euphorbia cyparissias), several small dead areas of dead and yellow plants were observed. In these areas the dead and yellow leaves of the affected plants were matted together by the hyphae of a species of Rhizoctonia.

A large irregular brown area also was observed in a neighboring planting of Corsican sandwort (Sagina subulata) (Fig. 1). A dark green margin about 1 inch wide was found around the edge of the brown area. Hyphae of a species of Rhizoctonia was growing profusely over the plants in the affected area.

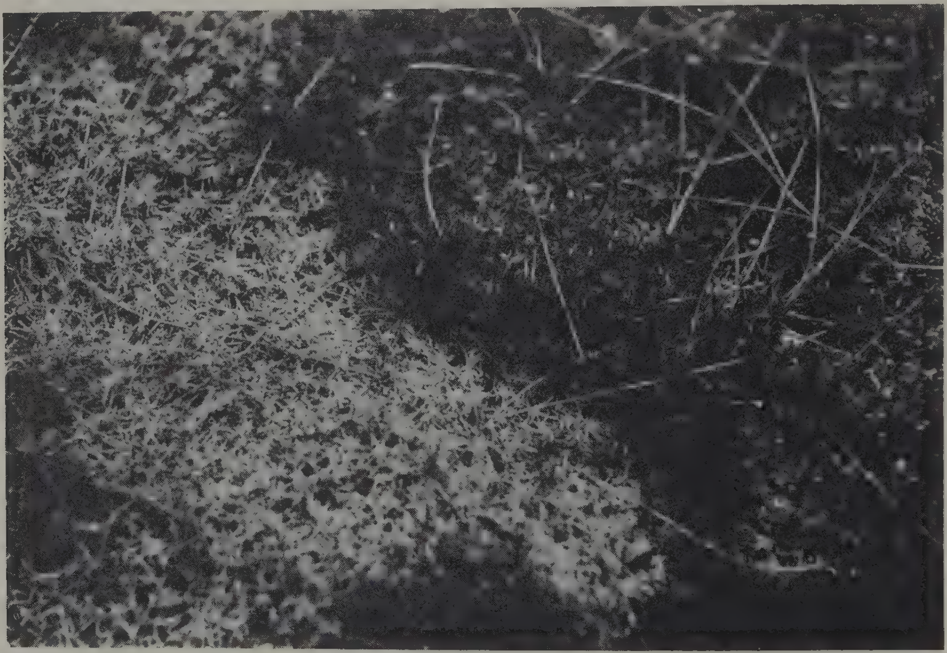

FIGURE 1. Corsican sandwort showing large irregular brown area with dark green margin.

Piper and Coe (1) reported in 1919 that common mouse-eared chickweed (Cerastium vulgatum) was very susceptible to the brown patch disease caused by Rhizoctonia solani Kuehn. R. solani has been reported as being the cause of damping-off of cuttings of florists' poinsettia (Euphorbia pulcherrima) (2). Kerr (3) reported severe injury to pearlwort (Sagina procumbens) by R. solani in Australia where pearlwort is grown as a lawn plant.

So far as can be determined, this is the first report of a species of Rhizoctonia on snow-in-summer, cypress euphorbia, and Corsican sandwort.

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[1] Associate Professor of Plant Pathology Extension, The Pennsylvania State University, University Park, Pennsylvania.

STUNT NEMATODES AND TOBACCO IN GUJARAT, INDIA

G. J. Patel, M. V. Desai, and H. M. Shah

When a tobacco grower in the village of Jakhla of Taluka Anand, Dist. Kaira complained of very subnormal growth of tobacco in his field during September 1961, the authors visited the field and found the crop to be very uneven. Some plants had reached the topping stage and some were of seedling size. The small plants had unduly thick and small leaves, although they had been in the field for over a month. Since conditions were good for plant growth during July, August and September and the field had been manured adequately, the very peculiar stunted growth of the plants suggested trouble with the roots. Stunted plants were uprooted very carefully from the affected spots. Normally root knots would be expected on the poorly developing plants in this area, but surprisingly not a single root knot was found on any of the plants examined and the nematode count of the soil on an average was 12 per 5-g sample. Moreover, the root development was very sparse and singular. Based on these observations, stunt nematode was suspected as the cause of the very subnormal growth.

Although the season was advanced, an experiment was initiated to test the effects of fumigation on this heavily nematode infested soil. The grower uprooted some plants, and on September 11, 1961 treatments began with fumigant 1,3-dichloropropene-1,2-dichloropropane (D-D). Fumigation was done in lines with a spacing of 3 feet, injecting 5 cc of D-D (about 6 inches deep) at each spot about 1 1/2 feet apart. Four replications were made and the plots contained two rows of 10 plants each. The original crop in the control plots was not disturbed, whereas in the fumigation plots the treatment was given after uprooting the plants and the crop replanted on September 27, 16 days after the fumigation treatment.

The experimental area was visited several times and the crop growth in the fumigated plots, though planted about 2 months late, was much superior to growth in control plots (Fig. 1). The crop was harvested on December 21 and green weight of leaves recorded (Table 1).

Table 1. Fumigation with D-D and green yield performance of bidi tobacco at Jakhla.

	Yield in grams per plot	
Replication	fumigated	control
I	4530	2220
II	4580	1650
III	5280	2400
IV	5730	1470
Mean	5030	1935

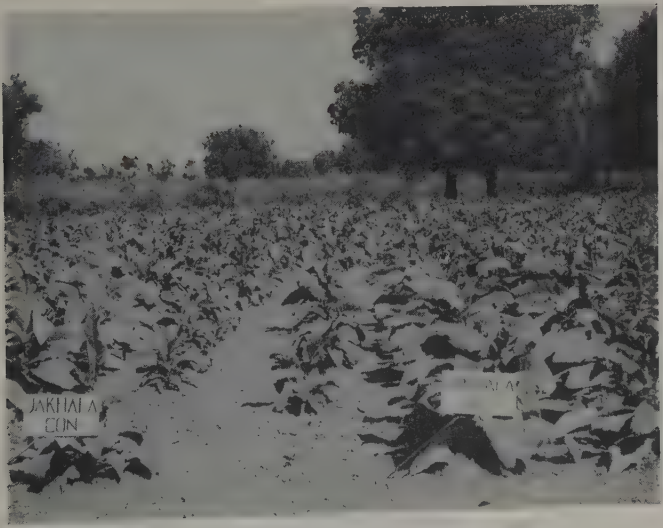

FIGURE 1. Fumigation of soil with D-D and plant growth.

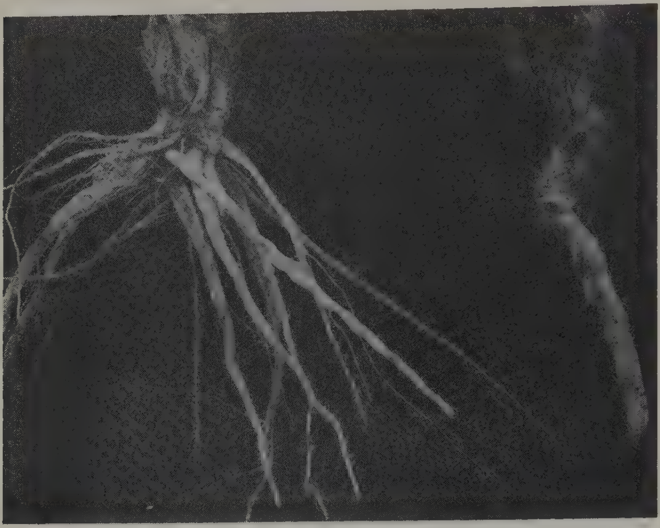

FIGURE 2. Sparse and singular root system in control plots (right).

The results show vividly the effect of spot fumigation with D-D, which is a widely used nematocide. In spite of the fact that the crop in fumigated plots was transplanted about 2 months later than the control plots and had unfavorable seasonal conditions, it had a yield increase of about 160%; undoubtedly this increase would have been much more if the experiment had been carried out during the normal season.

After harvest, the plants were dug out carefully from both the control and the fumigated plots. Root development in control plots was sparse and singular as compared with the large number of normal roots in the fumigated plots (Fig. 2).

The four factors of heavy infestation of the soil with nematodes, complete absence of root knots, very sparse and singular root development in control plots, and the peculiar stunted growth of the plants suggest that in all probability stunt nematodes were the cause of very subnormal growth of bidi tobacco crop at Jakhla. Further study is in progress.

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BACTERIAL SPOT DISEASE OF TOMATOES AND PEPPERS IN ISRAEL[1]

Zafrira Volcani

Bacterial spot disease, (Doidge) Dowson (1), has been found in Israel on tomato fruits since 1939 (2). Its causal agent, Xanthomonas vesicatoria, was identified, however, for the first time in 1950 (7).

The disease occurred mainly in the Coastal Plain during the spring, beginning of summer and autumn. It was also found occasionally, though sporadically, during the winter months. It was not observed in the Jordan Valley or the Negev region, and its incidence was in general rather low. The disease did not until recently present a serious problem to the grower.

During the spring and the beginning of summer 1960 spot disease appeared in different areas of the country with an unusually high incidence on the commonly and newly grown "Marmand" and "Money-maker" varieties, respectively. For the first time leaves and stems were infected in addition to fruits. After a break during the hot summer months disease spread was renewed during the autumn. The disease caused serious damage in some places, particularly in the Coastal Plain and Western Galilee. It occurred during the spring and the beginning of summer of 1961, again causing serious damage.

During the autumn of 1961 the disease was detected for the first time in Israel on sweet pepper (Capsicum annuum). A moderately high incidence of leaf-spot lesions appeared in several fields on the variety "Gamba." The fruits, however, were not affected.

Positive results were obtained in direct inoculation tests with isolates and respective reisolates of a yellow-pigment producing bacterium on fruits, leaves and stems of "Marmand" and "Money-maker" tomato varieties, as well as on leaves of sweet and red pepper.

Similar results were obtained in cross inoculations with both tomato and pepper isolates. No significant differences were observed among the various isolates with regard to rate and size of infection. On the other hand, the pepper organisms differed distinctly from the tomato isolates in their delayed fermentation reaction with a number of carbohydrates. Slight differences were also observed among the various tomato isolates with regard to their growth and one or two of their physiological characters. The bacterial isolates and their reisolations were identified as Xanthomonas vesicatoria (Doidge) Dowson (2, 3, 4) owing to the close similarity between the former and the latter organism.

From the results of inoculation tests it appears also that the disease can develop at relatively high temperatures (33° to 35°C during the day) if relative humidity is high (3, 5). But the progress of infection is arrested at the same temperatures, under relatively low humidity. Day temperatures above 33° to 35°, even at relatively high humidity levels, inhibit its development. Sprinkler irrigation undoubtedly favors the development and spread of the disease in the field (6), whereas the Hamsins (extreme dry conditions) have an arresting effect.

Further studies of the disease and its control under conditions prevailing in Israel are in progress.

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NATIONAL AND UNIVERSITY INSTITUTE OF AGRICULTURE, REHOVOT, ISRAEL

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CURRENT-SEASON AND TUBER-PERPETUATED SYMPTOMS OF POTATO CURLY TOP

Golden L. Stoker and Orson S. Cannon[1]

Summary

Symptoms of current-season curly top infection as it occurs in Utah are fairly constant and differ from other potato diseases that commonly occur in Utah. Outstanding characteristics of current-season curly top are dwarfing; yellowing, elongation, and a rolling upward from the midrib of the terminal leaflets; and marginal yellowing, elongation, cupping, rolling, twisting, bulging, and roughening of the leaflets near the growing point, with the midribs and veins developing a rough, irregular, knotted appearance. In Utah, the name haywire has been used in referring to plants exhibiting these symptoms.

Symptoms of tuber-perpetuated curly top are extremely variable and are influenced by the environment under which the potatoes are grown. Delayed emergence and dwarfing are severe under all environments, with the shoots often being so short they fail to emerge from the soil. When grown in the greenhouse at Logan, Utah and at Oceanside, California during the winter months, the dwarfed plants had a dark green color characteristic of curly top symptoms described as green dwarf. Under field conditions in Utah, the color of the dwarfed plants varies from normal green through light green to yellow; and diseased plants range from extreme dwarfing to symptoms resembling those of current-season infection.

The curly top virus has been known to cause disease in potatoes since 1925 (9), but there has been confusion regarding the symptoms. This paper is an effort to clarify the symptom picture, especially as related to the disease in Utah, where it has been observed by the senior author over the past 16 years while examining fields for seed certification.

Severin (9), who first reported curly top of potato, stated that "potato plants naturally infected with curly top were stunted, with yellowish, inward-rolled leaflets." He did not mention tuber-perpetuated symptoms. Jones, Vincent, and Burk (5) stated that curly top "showed as dwarfing and erectness of the plants, rolling, harshness, and reddening of the foliage, and extreme dwarfing and increase in the number of tubers." According to them, the symptoms resembled those associated with psyllid yellows or aster yellows. They described greenhouse tuber-transmitted symptoms that developed on three plants as: "showing spindly sprouts, erectness, inward rolling, and yellowing of foliage." In 1946 Milbrath (8) described green dwarf, which later was shown by Menzies and Giddings (6, 7) to be caused by the curly top virus. Milbrath described tuber-perpetuated and current-season symptoms and stated that current-season symptoms are "very similar to the symptoms of the terminal growth of tuber-perpetuated green dwarf." Menzies and Giddings (7) observed that current-season symptoms on the Russet Burbank variety are quite distinctive under eastern Washington conditions. They described ruffled "pinched in" terminal growth, with the plant becoming stunted and having stiff, erect, leaf petioles and lateral shoots, and a color changing from normal to a pale olive green. They stated that "tuber-transmitted symptoms vary widely, particularly where seedling varieties are concerned and are readily confused with those of other diseases." They further stated that dwarf shoots may be green or display ranges of yellow, red, and bronze pigmentation. Their descriptions of seed piece dormancy and very dwarfed shoots agree with those given by Milbrath. Gardner (1) reported that the disease called "haywire" in Utah is caused by the curly top virus. He showed pictures of tuber-perpetuated curly top and stated symptoms were "well described by Severin for curly top in California, Milbrath for green dwarf, Menzies and Giddings for curly top in Washington, and Goss for 'haywire'."

Critical examination of the descriptions given in the foregoing papers reveals that curly top symptoms may be extremely variable in different localities and with different varieties. In Utah, however, the authors have observed that current-season symptoms are fairly constant and differ from those of other diseases that commonly occur in Utah, while tuber-transmitted symptoms are extremely variable and may have little or no resemblance to current-season symptoms. Current-season curly top symptoms of potatoes, known as haywire in Utah, are similar to haywire symptoms as originally reported from Nebraska by Goss (3) and more completely described and illustrated by Jensen and Livingston (4); however, the present authors did not study Nebraska haywire to determine its relationship to the current-season curly top symptoms called haywire in Utah.

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Current-season Symptoms: Symptoms first appear at the apex or terminal growth of the plant. Symptoms may or may not appear on all stems of a plant simultaneously, and they may appear at any time during the development of the plant. Terminal growth is markedly reduced, and this results in a stunting of the plant. Disease symptoms appear only on the parts of the plant that grow after infection occurs.

Leaflets of the terminal leaves are erect, stiff, rolled upward from the midrib and often are curled over the rachis (Fig. 1). Green color development is inhibited and the growing point is light green to yellow in color. The cluster of small, rolled, terminal leaflets with their pubescent undersides exposed gives the terminal growth a greyish white appearance.

Leaves near the growing point are longer in proportion to width than healthy ones, and basal leaflets are much more stunted than terminal leaflets (Fig. 1). The margins of leaflets become light green to yellow and the affected leaflets may fade to a light green or yellow between the veins. Some leaflets become distorted and curve sideways, some may roll slightly upward and some downward. The marginal chlorotic tissue often is restricted more in growth than is the central greener tissue, causing a cupping or an upward or downward bulging of the leaflets (Figs. 2 and 3). They have a thick, rough or wrinkled appearance as if gathered along the midrib. The midribs, and to a lesser extent the veins, often develop a rough, irregular, knotted appearance (Fig. 3). Central green portions of the leaflet may continue to grow and cause a bulging of the tissue between the veins, thus giving the leaflets a rough appearance which, on a miniature scale, resembles mountainous terrain with the veins appearing as streams or ravines (Fig. 4). The leaves of plants infected early in the season may lose all green coloration by late summer and what earlier were chlorotic margins may become necrotic.

All varieties of potatoes grown in Utah develop the characteristic curly top symptoms when naturally infected in the field with the curly top virus; however, the Kennebec variety is much more severely damaged than are the Russet Burbank or White Rose varieties. The degree of marginal and interveinal yellowing, leaf distortion, and necrosis varies slightly with varieties. The thicker leaflets of the Kennebec variety tend to remain smoother, and their marginal yellowing may be less pronounced as the leaflets fade to a light green or yellow color. The margins and tips of leaflets of red tuber varieties may develop a reddish or purplish color.

Plants that develop disease symptoms in the early stages of growth produce a few small, misshapen tubers. Large plants that develop symptoms later in the growing season may produce one or more marketable tubers in addition to small ones which often are misshapen. Frequently, the large tubers are misshapen.

Tuber-perpetuated Symptoms in the Greenhouse and at Oceanside, California: Tubers from plants growing in commercial fields with typical Utah haywire (current-season curly top infection) were planted in the greenhouse at Logan, Utah, and in the field at Oceanside, California in December. Kennebec, Russet Burbank, and White Rose tubers were planted in the greenhouse while the Oceanside planting was limited to the White Rose variety. At both locations, plants developed green dwarf symptoms as described by Milbrath (8). Within 103 days after planting at Oceanside, California 25.1% of the tubers had not produced emergent plants. Of the tubers involved, 19.8% produced plants that exhibited green dwarf symptoms, 15.4% produced smaller plants with no definite symptoms, and 39.7% produced large, normal appearing plants.

The tendency of the Kennebec variety to be more severely damaged in the field by current-season curly top than the Russet Burbank and White Rose varieties was also observed with tuber-perpetuated curly top (Table 1). Over 97% of the seed pieces planted in the greenhouse from tubers produced on diseased Kennebec plants either failed to grow or produced severely stunted plants. The corresponding percentages for the Russet Burbank and White Rose varieties were 51 and 56 respectively. The dark green, stunted plants emerged over a period of several weeks. Some shoots were so short they failed to reach the soil surface. Of the three varieties, 33.7% of the Russet Burbank, 40.5% of the White Rose, and 59.1% of the Kennebec sets produced plants that remained extremely dwarfed, with the leaves of the growing points pinched together in a cluster of small leaves. After a delayed early growth, some plants developed as tall, dark green, upright plants with a single large stem. Slightly less than 18.0% of the Russet Burbank and White Rose, and 0.7% of the Kennebec sets produced healthy plants. Sets of some tubers produced both diseased and healthy plants, which is evidence that the virus did not occur uniformly through the tuber.

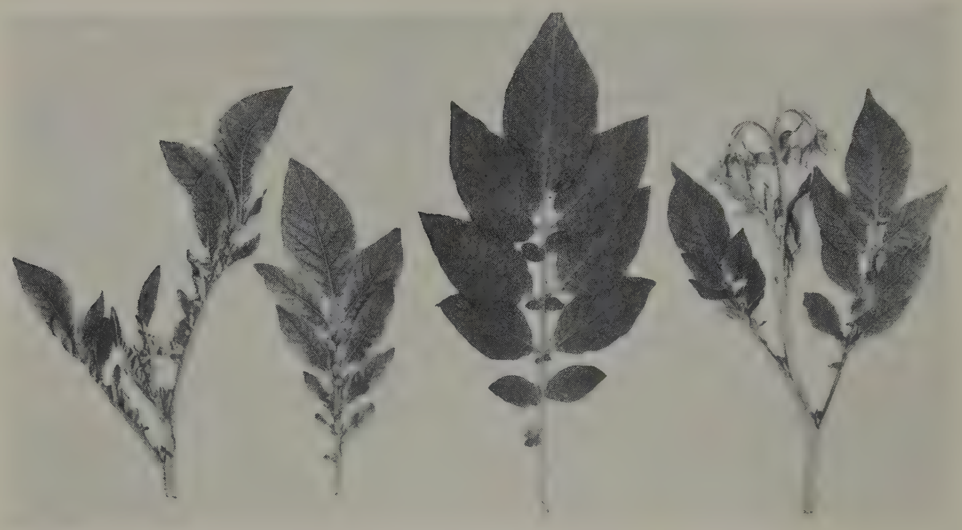

FIGURE 1. Terminal growth and leaf of Russet Burbank potato showing current-season curly top symptoms (left) and similar parts of healthy plant (right). Note cluster of small, rolled, elongated terminal leaflets with pubescent underside of leaflets exposed.

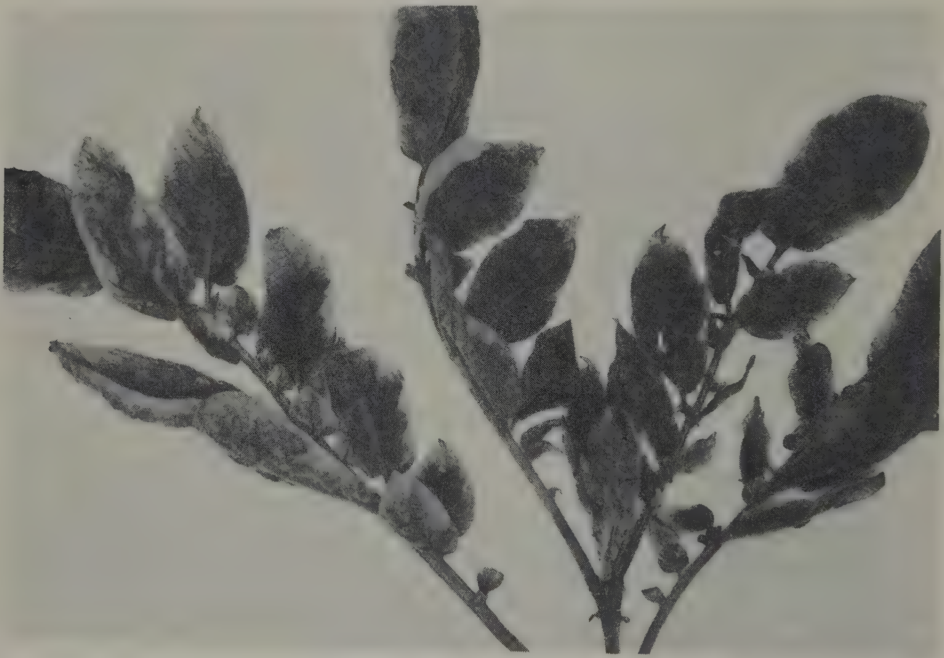

FIGURE 2. Top portion of Russet Burbank plant infected with current-season curly top. Note curved, rolled, bulged, or distorted leaflets with faded margins.

FIGURE 3. Healthy Russet Burbank leaf (right) and diseased leaf (left). Note elongated, rough, or wrinkled leaflets with faded margins and rough, irregular knotted midribs and veins.

FIGURE 4. Leaflet showing green central portion with bulging tissue between veins.

FIGURE 5. Tuber-perpetuated curly top symptoms. White Rose plants grown from tubers harvested from plants exhibiting current-season curly top symptoms.

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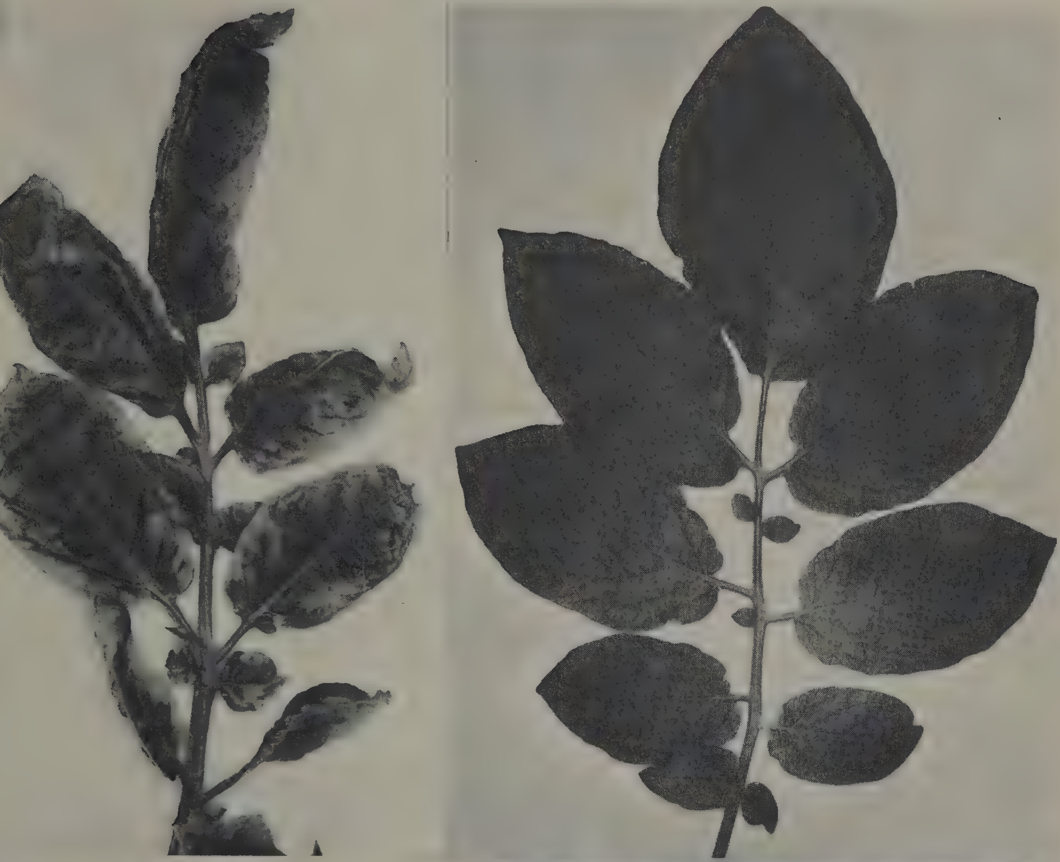

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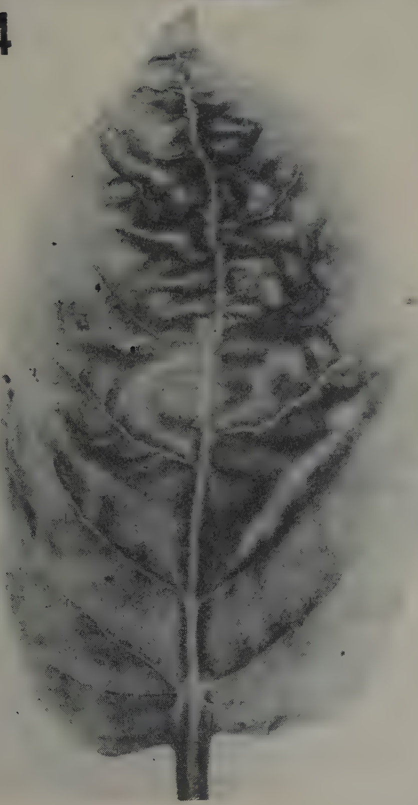

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Table 1. Tuber-perpetuated curly top. Growth in the greenhouse from tubers produced by plants naturally infected with curly top virus.

Variety	Sets planted	No growth		Severely stunted		Slightly stunted		Healthy	
		no.	%	no.	%	no.	%	no	%
Russet Burbank	297	53	17.8	100	33.7	91	30.6	53	17.8
White Rose	148	23	15.5	60	40.5	39	26.4	26	17.6
Kennebec	149	57	38.3	88	59.1	3	2.0	1	0.7

Table 2. Tuber-perpetuated curly top. Growth in the field from tubers produced by plants naturally infected with curly top virus.

Variety	Tubers planted	Plants emerged after 112 days	% emergence
Kennebec	291	88	30.2
White Rose	113	45	39.8

Typical curly top symptoms developed on tomato plants that were grafted onto potato plants exhibiting green dwarf symptoms in the greenhouse.

Tuber-perpetuated Symptoms in the Field at Logan, Utah: Kennebec and White Rose tubers harvested at the same time as those planted in the greenhouse were saved for planting in the field the following spring. One hundred twelve days after planting, many tubers had not yet produced sprouts long enough to emerge from the soil (Table 2). Most of the non-emergent plants developed short, thick sprouts that did not grow further. Those plants that did emerge ranged from extreme dwarfing (Fig. 5) to normal growth. Many of the plants had the characteristic green dwarf growth, but instead of being dark green, the color ranged from light green to yellow, while a few plants developed symptoms similar to those of current-season infection.

When tomatoes were grafted onto potato plants exhibiting any of the tuber-perpetuated symptoms, typical curly top symptoms resulted in the tomatoes.

Tuber-perpetuated Symptoms Expressed in the Second Generation: If plants produced tubers in the greenhouse, these were harvested, stored to break the rest period, and then planted in the field. Again in the field there was a very marked delay in germination. Sixty days after planting, 20.5% of the Russet Burbank, 40.8% of the White Rose, and only 3.2% of the Kennebec had emerged. Eventually (after 112 days) the percentages were 83.4, 79.6, and 10.5 for the Russet Burbank, White Rose, and Kennebec respectively.

In addition to non-emergence and late emergence, some of the second generation plants expressed tuber-perpetuated curly top symptoms, and the curly top virus was transmitted to tomatoes by grafting.

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UTAH AGRICULTURAL EXPERIMENT STATION, LOGAN

INJURY TO SOYBEANS CAUSED BY PRATYLENCHUS ALLENI[1]

Virginia R. Ferris and R. L. Bernard[2]

Summary

Soybean plants grown in the greenhouse in the presence of Pratylenchus alleni, a species originally discovered in soybean breeding plots, were found to have their root weight reduced 25%. In one experiment this reduction was obtained at a level of infection comparable to that of field-grown plants. It is considered likely that under certain environmental conditions infection with this nematode would cause reduced growth and yield of field-grown soybean plants.

A large number of species of plant parasitic nematodes were observed by the authors (2) to be commonly present in the soybean field soils used for breeding experiments. Occasionally a species builds up to high numbers in a given field. It would be desirable to know whether these nematodes have the capabilities of becoming pathogenic to the crop and how much injury is likely to occur. Published information on nematode injury to soybeans appears to be limited to work with the genera Heterodera and Meloidogyne.

Recent work such as that of Perry, et al. (4) on the nematode-induced disease known as "summer dormancy" of blue grass in Wisconsin has pointed up the importance of the environment in the manifestation of a disease primarily characterized by root injury. This disease does not become noticeable in cool moist weather, but only if soil moisture becomes limiting. It is possible that a similar situation could exist in a field crop such as soybeans. In experimental breeding areas, particularly, the potentialities for injury by the nematodes likely to be encountered should be understood.

During a nematode survey in Illinois soybean breeding plots in the summer of 1958, a single undescribed species of Pratylenchus was recovered in large numbers from several varieties of soybeans (Table 1). Subsequently it was described by the senior author as Pratylenchus alleni (1). In the meantime experiments were begun to maintain the nematode in pot culture and to determine, if possible, the extent of damage to soybean plants of an infestation comparable to that found in field plants.

Table 1. Pratylenchus alleni emerged from roots of field-grown soybean plants.

Variety	Number per gram
Clark	733
Harosoy	843
Kent	515
Lincoln	1914
Lindarin	954
Shelby	849
C1128[a]	1132

[a] An experimental strain.

MATERIALS AND METHODS

Inoculum was prepared in the following manner. Soil from the field originally found to be infested was planted to soybeans. After 3 months, roots from these soybean plants were removed from the pots, washed well, and added to sterile soil in clean pots. These pots were planted with soybeans of the Lincoln variety.. The roots from these infected greenhouse-grown plants were washed, blotted, and cut into 2-inch lengths.

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[1] Publication No. 363 of the United States Regional Soybean Laboratory, Urbana, Illinois.

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In the first test to determine pathogenicity four plants of the soybean variety Lincoln were grown in each of twenty 6-inch pots with potting mixture, consisting of equal parts of field soil and sand, sterilized by treatment with methyl bromide. Half-gram lots of inoculum were added to each of 10 pots at the time of planting. Two additional lots of inoculum were used to determine the approximate number of nematodes that would emerge from this quantity of roots. The remaining 10 pots were maintained as uninoculated checks.

The plants were allowed to grow in the greenhouse for 3 months. Then the soil in each pot was washed gently from the roots. The excess water on the roots was removed by low-speed centrifugation after the method described by Linford and Rhoades (3). They were then weighed. Each group of four root systems from a single pot was weighed together as a unit. One-half gram of roots was taken from each unit for determining the number of nematodes emerging from the roots.

Uniform procedures were followed in the extraction of nematodes from the roots of all plants. Three grams of roots were incubated from the field plants and 1/2 gram from each pot of greenhouse plants. The weighed roots were placed in a quart jar with a small amount of water and the lid placed loosely on the jar. All jars were maintained at 68° to 70° F. After 24 hours a small volume of water was sprayed over the roots and then poured off into a 150-ml container. This step was repeated until the container was full (approximately 10 washings). The roots were then rinsed in the jar with 2 quarts of clean water, which was then discarded. Three additional such collections at 2-day intervals were made from each sample of roots. The collections were then combined and counts were made on two aliquots per sample.

At the conclusion of the first experiment with the variety Lincoln, a second was begun with the variety Adams. It was conducted in a manner similar to that described for the first test and also included 20 pots, or 80 plants. The weight of each bundle of roots used to provide inoculum for this experiment was 1.8 grams.

RESULTS AND DISCUSSION

The frequency distributions of the weights of each group of four root systems from a single pot are given in Figures 1 and 2 for the two tests. The roots from the pots to which nematodes had been added weighed an average of 25.5% less in the first test and 25.2% less in the second test than those of the check pots. An analysis of variance based on pot-to-pot variability showed this difference to be highly significant in both tests. The numbers of nematodes emerging from the roots of the first test ranged from 354 to 2394 per gram of roots, with an average of 1350. These figures are comparable in magnitude to those obtained for nematodes emerging from the field plants originally found to be infected (Table 1). The numbers of nematodes recovered from the roots of the infected plants of the second test ranged from 1060 to 8380 per gram of roots, with a mean of 3650. An average of 47,000 nematodes emerged from the bundles of the roots similar to those used as inoculum for the first test, and 5000 nematodes from those of the second test. No nematodes emerged from the roots of the check plants in either test.

The roots of the inoculated plants in both tests felt limp and were dark brown as a result of the many small brown lesions, typical of Pratylenchus injury. The root systems of the check plants felt springy and were light in color. The inoculum for these tests (infected roots from greenhouse-grown plants) was, of course, non-sterile. Under such conditions there is always the possibility of unknown side effects from other microorganisms, as is pointed out by Mountain (5). Because of this fact, frequent stained preparations were made of the roots. Dissection of these preparations always showed a nematode in some stage of development to be present in the root near a brown lesion. No other microorganisms, including fungal hyphae and spores, were seen in the roots. The inoculum was monospecific with respect to nematodes. Therefore it seems probable that the reduction in root weight observed in the inoculated plants was the result of the pathogenic action of P. alleni.

There were no noticeable differences in top growth of inoculated and check plants. It must therefore be concluded that in these greenhouse experiments the amount of root growth was not a limiting factor. However, the experiments do demonstrate that a marked reduction of root development in soybean plants can occur in the presence of P. alleni. In one of the tests this reduction was accomplished at a level of infection at least comparable to that observed in field-grown plants in naturally infested soil. Under field conditions when either water or nutrients are limiting, such a reduction in root growth would probably be reflected in reduced growth and yield of the plant as a whole.

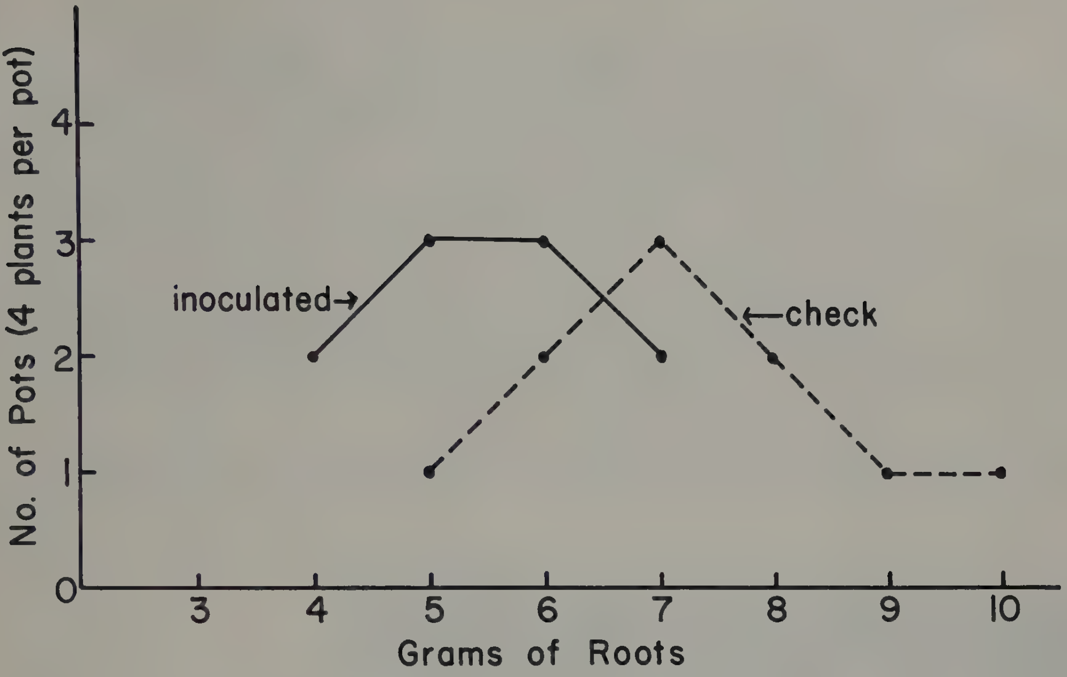

FIGURE 1. Frequency distributions of root weights of infected and non-infected soybean plants, variety Lincoln.

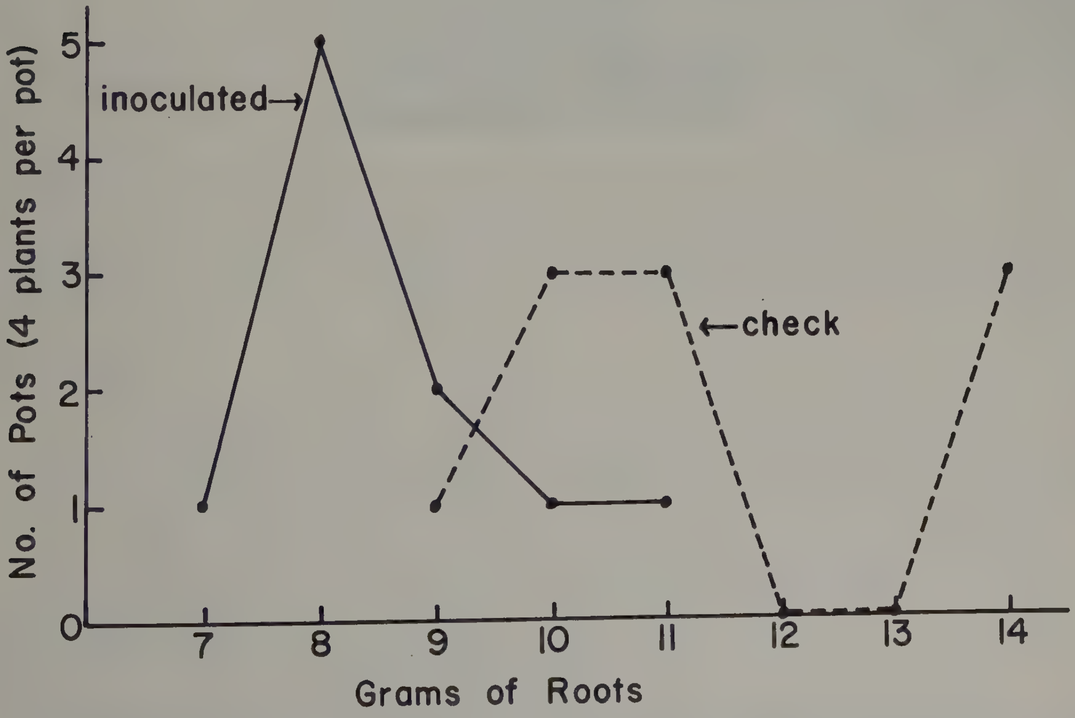

FIGURE 2. Frequency distributions of root weights of infected and non-infected soybean plants, variety Adams.

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OCCURRENCE OF CITRUS SOUR ROT IN LOUISIANA

J. B. Sinclair, N. L. Horn, and R. T. Brown[1]

Sour rot of citrus, caused by *Geotrichum candidum* Lk. ex Pers. emend Carmichael (1), was first reported in Louisiana citrus groves during the late summer and fall of 1957 (2, 3). The disease was originally observed on Washington navel orange (*Citrus sinensis*) and later on Satsuma (*C. nobilis* var. Owari) and kumquat (*Fortunella* sp.). Reported losses at that time ranged from 10 to 30% of the crop. The disease has been observed on Louisiana citrus each season since 1957, but losses were not significant.

Sour rot reached epiphytotic proportions again on Louisiana citrus during December 1961. An estimated 20,000 bushels of Satsuma oranges alone were lost in Plaquemines Parish, the chief citrus growing parish of the State, besides a lesser number of bushels of Washington navel oranges. At the Plaquemines Parish Experiment Station, Port Sulphur, it was estimated that 400 bushels of Satsumas and 300 bushels of navels were lost. Most of the Satsuma crop had been harvested, but all fruit of this type remaining on trees in the field were lost.

The disease appeared during the second week in December 1961 after an extended period of warm, rainy and foggy weather. The rot appeared on fruit, while still on the trees, as a watery, sour rot. Rotted fruit first showed a water-soaked spot, which rapidly enlarged and soon involved the entire fruit. The white mycelium and spores of the fungus appeared on infected tissue in a few days. As the entire fruit became rotted, it took on a pyriform shape before dropping to the ground (Fig. 1).

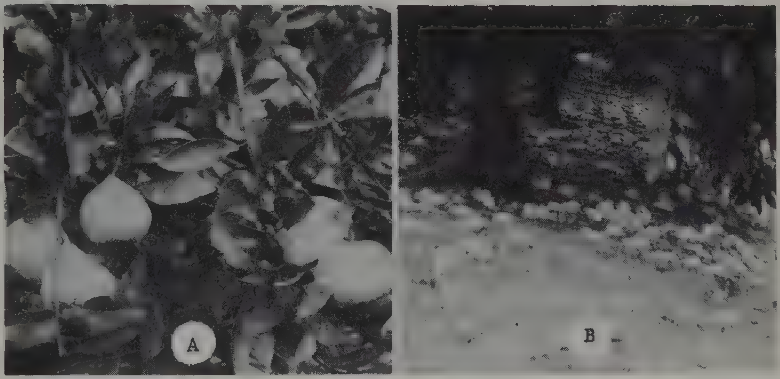

FIGURE 1. Sour rot of citrus. A -- Rotted fruit on the left take on a pyriform shape; nonrotted fruit on the right. B -- Fruit drop as a result of infection by *Geotrichum candidum*.

The rot was observed to be more serious on Satsuma and mandarin-type citrus than on other citrus. This may have been due to the fact that most of the former types were fully ripe, while other citrus, like Washington navel and Valencia, (*C. sinensis*) were still green or just beginning to ripen. It was noted that those Satsuma growing on rootstocks that produced a denser foliage had more rotted fruit than trees on rootstocks that produced a less dense foliage.

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THE EFFECT OF VARYING DEGREES OF ROOT KNOT INFESTATION ON WINTER INJURY TO THE PERENNIAL CARYOPTERIS

J. D. Wilson[1] and Orve K. Hedden[2]

Summary

In the spring of 1961, a wide variation existed in the degree of winter survival of Caryopteris plants in plots that were treated "preplant" with different nematocides in the spring of 1959. In the fall of 1960 the data on control of root knot, caused by Meloidogyne hapla, was taken in these plots and the results published a short time later. Most, but not all, of the plants in these plots were harvested and sold by the grower in 1960. It was in the unharvested portion of the plots that the differences in winter survival were evident. In those plots where root knot control was rated as good in 1961 the percentage of plant survival over the winter of 1960-61 was from 50 to 90%. However, nematode control was poor, winter killing was severe, the survival being as low as 9% in the untreated check. In a single row that received a side-dressing treatment of Fumazone in June 1960 the added control of root knot increased survival in the untreated check plot from 9 to 39%, and this postplant treatment also reduced winter injury in most of the treated plots as well.

INTRODUCTION

Comparatively few references in nematological literature deal with the possible relationship between root damage by nematodes and winter injury, and still fewer involve specific reference to a relationship between root knot and winter survival. Edgerton and Parker (1) reported on the effect of damage by the lesion nematode (Pratylenchus penetrans) on cold hardiness of Montmorency cherry trees, where a scarcity of feeder roots on heavily infested rootstocks increased winter injury by delaying fall maturity of twig growth. However, a direct connection between root damage by a Meloidogyne species and winter injury has seldom been established.

Table 1. Relationship between degree of root knot infestation in the fall of 1960 and the degree of survival of Caryopteris during the winter of 1960-61.

Treatment[d]	Rate/acre (1959)	% plants surviving 1960-61 winter[a]	% survival, single row side-dressed with DBCP in 1960	Root knot score Oct. 1960 (0 = none, 4 = maximum[b])
Nemagon granules (17.3%)	300 lb	90	99	1.0
Nemagon EC-2 (50%)	4 gal.	72	96	2.6
Nemagon EC-2 (50%)	3 gal.	70	45	3.0
Nemagon granules (17.5%)	200 lb	66	72	2.8
Dorlone	24 gal.	54	90	2.8
EDB (M731) (73%)	8 gal.	40	75	3.4
D-D Emul.	30 gal.	39	24	3.2
Telone	30 gal.	27	39	3.8
D-D	30 gal.	21	--[c]	3.2
C-9882 (60% Em.)	5 gal.	11	--[c]	3.8
Untreated Check	---	9	39	3.8
Cynem (4#/gal.)	4 gal.	6	63	3.8

[a] One hundred plants counted.
[b] These data taken from a previously published paper (3).
[c] This portion of the only remaining side-dressed row had been harvested.
[d] EDB = ethylene dibromide; D-D = 1,3-dichloropropene-1,2-dichloropropane; Telone = 1,3-dichloropropene (Tech.); C-9882 = diphenyl sulfide; Cynem = O,O-diethyl O-2-pyrazinyl phosphorothioate.

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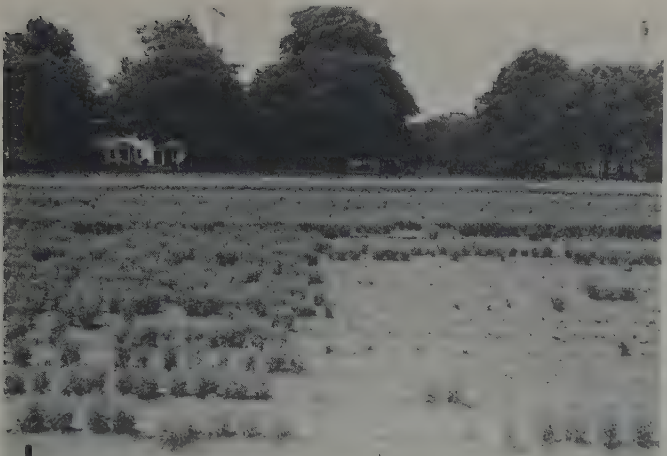

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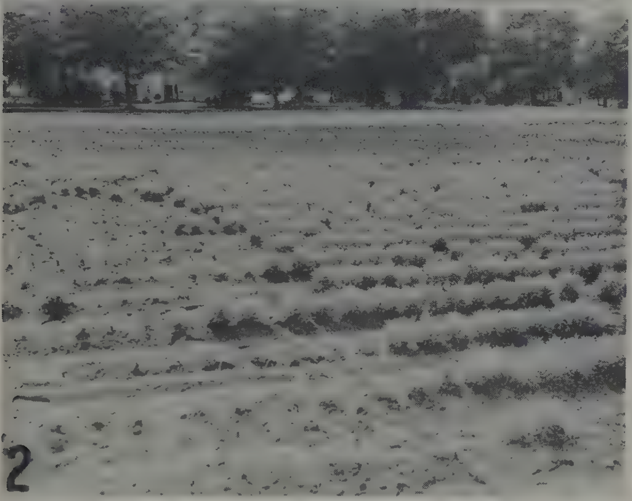

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FIGURE 1. Comparative degree of survival from winter injury (1960-61) in plots that received 4 gallons of Nemagon EC-2 in May of 1959 (left) and in an adjacent plot that was treated with a material that gave much poorer control of root knot infestation.

FIGURE 2. Comparative survival of Caryopteris in a plot that received 4 gallons of Nemagon EC-2 in 1959 (right) and in an untreated check plot. The string marks the original boundary between the two plots.

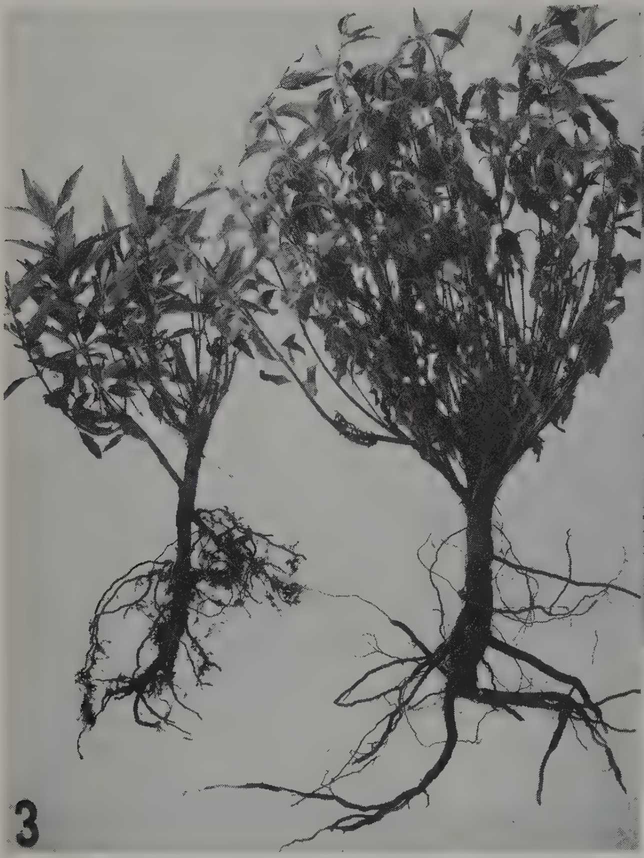
In the spring of 1959 a number of nematocidal formulations were applied as preplant treatments to an area to be planted a short time later to *Caryopteris incana*. Most of the plants originally set in the variously treated plots were dug in the fall of 1960, when the degree of root knot infestation was estimated (3). Preliminary tests made in the spring of 1960 indicated that control of root knot, caused by *Meloidogyne hapla*, had not been very good in several instances and several rows of plants were treated with a side-dressing of 1,2-dibromo-3-chloropropane (DBCP) (Fumazone) (2).

Because not all of the plants in the experimental block could be disposed of by the nursery in the fall of 1960, several rows were not dug; this afforded an unexpected opportunity in the spring of 1961 to observe an apparent relationship between the degree of root knot infestation and winter injury.

RESULTS AND DISCUSSION

Since the lines of demarcation between survival and apparent death of the plants were so clear cut (Figs. 1 and 2), the original plot boundaries were established and then the living and dead plants present in the areas that had been treated with different nematocidal formulations in the spring of 1959 (3) were counted. From these data the survival percentages shown in Table 1 were calculated. Several living and dead plants were dug from the untreated check plot as well as from two or three of those showing a high percentage of living plants. Figure 3 shows the comparative root condition of the living plants from the check plot and from the one originally treated with 300 pounds of 17.3% DBCP (Nemagon) granules (3). Some of the infested plants did survive the winter. However, virtually all of the dead plants in any and all plots showed a high degree of infestation by root knot, whereas most of those that were alive in plots where survival was above 50% were comparatively free from root knot.

In every plot but one (that were treated with a mixture of 75.2% of 1,3-dichloropropene and 18.7% ethylene dibromide (Dorlone) in 1959) where survival exceeded 50% of the plants present, the plots had been preplant-treated in the spring of 1959 with DBCP (Nemagon). In only one plot where the severity score of root knot was rated as high as 3.0 (on a scale of 0 = none and 4.0 = the maximum degree of infestation) in the fall of 1960 was survival in excess of 50%, and when the severity rating was as high as 3.8 survival was 27% or less.



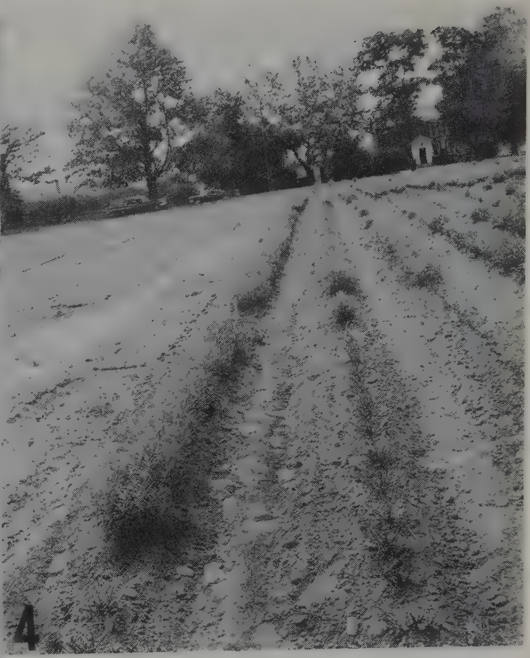


FIGURE 3. Comparative degrees of root knot infestation and root growth in the spring of 1961 on plants taken from the untreated check plot (left) and from the plot treated preplant with 300 pounds of 17.5% Nemagon granules in the spring of 1959. Ninety percent of the latter survived the winter of 1960-61 whereas less than 10% of the check plants showed new growth in 1961.

FIGURE 4. Effect of a side-dressing treatment of Fumazone applied in the spring of 1960 on winter survival in 1961. The three rows in the foreground are in the untreated check plot and the next two more distant plots received Dorlone and Nemagon, respectively, in 1959. In the check plot the row on the left was side-dressed with Fumazone in 1960, whereas the second and third toward the right were not.

Another interesting feature of the survival data concerns a single row, crossing all 1959 plots (3), that was side-dressed in the spring of 1960 with DBCP. This extra treatment in the second year of Caryopteris growth greatly reduced the degree of root knot infestation present in most of the plots that received a preplant treatment, and increased the percentage of winter survival in all plots but two; in some instances the increase was quite striking (Fig. 4).

Not only was the percentage of plant survival higher with better root knot control, but the surviving plants were much larger and bloomed more profusely during the summer of 1961.

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DUTCH ELM DISEASE IN KANSAS IN 1961[1]

C. L. Kramer[2] and Hugh E. Thompson[3]

During the past 5 years an annual report has been published on the spread of Dutch elm disease in Kansas. In the first article, two Kansas counties, Wyandotte and Johnson, included in the area of greater Kansas City were reported as having the disease in 1957 (4). By 1958 the disease had spread north into Leavenworth County and south into Miami County (1). In 1959 the disease was found to have continued its spread, moving west into Douglas, Franklin, and Lyon counties. In addition, positive locations were found in four cities in Cherokee and Montgomery counties in the southeastern corner of the State (2). Infection of those areas was apparently effected by spread from the Joplin, Missouri area, some 20 miles east of Cherokee County.

Although numerous samples were tested from the 29 counties separating the known areas of infection in the north from those in Montgomery and Cherokee counties in the south in 1959, it was not until the following year, 1960, that the disease was found in this area including Anderson, Linn, Allen, Bourbon, Neosho and Crawford counties. In addition, two counties in the northern quarter of the State, Shawnee and Atchison, were reported to contain the disease in 1960 (3).

During the past season, 1961, the disease has been located in 12 new counties, the greatest spread of any year since the disease has been known in Kansas. This brings the total to 29 counties that have now been reported as having positive locations of the disease. The new counties are Doniphan, Jefferson, Osage, Morris, Coffey, Woodson, Greenwood, Butler, Wilson, Elk, Cowley and Labette. Counties in which the disease is present are indicated in the map (Fig. 1) by a number denoting the year in which it was first found.

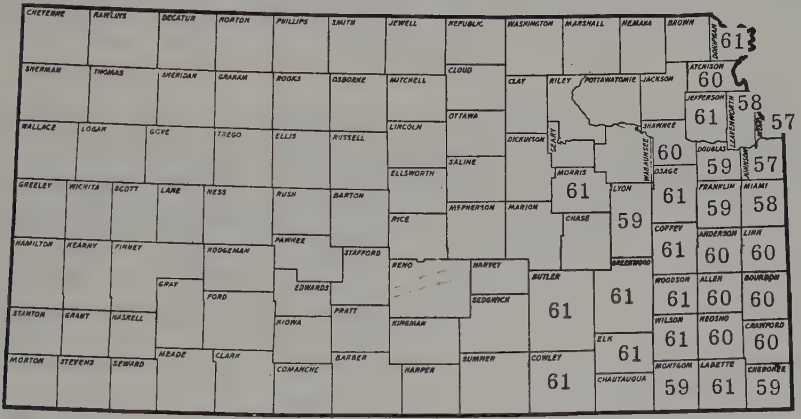
CHEYENNE
RAWLINS
DECATUR
NORTON
PHILLIPS
SMITH
JEWELL
REPUBLIC
WASHINGTON
MARSHALL
NEMAHA
BROWN
DONIPHAN 61
SHERMAN
THOMAS
SHERIDAN
GRAHAM
ROOKS
OSBORNE
MITCHELL
CLOUD
CLAY
RILEY
POTTAWATOMIE
JACKSON
ATCHISON 60
JEFFERSON 61
LEAVENWORTH 58
WYANDOTTE 57
WALLACE
LOGAN
GOVE
TREGO
ELLIS
RUSSELL
LINCOLN
OTTAWA
DICKINSON
GEARY
WABAUNSEE
SHAWNEE 60
DOUGLAS 59
JOHNSON 57
ELLSWORTH
SALINE
MORRIS 61
OSAGE 61
GREELEY
WICHITA
SCOTT
LANE
NESS
RUSH
BARTON
LYON 59
FRANKLIN 59
MIAMI 58
RICE
MCPHERSON
MARION
CHASE
COFFEY 61
ANDERSON 60
LINN 60
HAMILTON
KEARNY
FINNEY
HODGEMAN
PAWNEE
STAFFORD
RENO
HARVEY
GREENWOOD 61
GRAY
FORD
EDWARDS
BUTLER 61
WOODSON 61
ALLEN 60
BOURBON 60
STANTON
GRANT
HASKELL
PRATT
SEDGWICK
KIOWA
KINGMAN
WILSON 61
NEOSHO 60
CRAWFORD 60
ELK 61
MORTON
STEVENS
SEWARD
MEADE
CLARK
COMANCHE
BARBER
HARPER
SUMNER
COWLEY 61
CHAUTAUQUA
MONTGOMERY 59
LABETTE 61
CHEROKEE 59

FIGURE 1. Distribution of Dutch elm disease in Kansas, 1961.

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THE INFLUENCE OF TIME OF PLANTING AND RUNNER REMOVAL ON THE INCIDENCE OF VERTICILLIUM WILT IN STRAWBERRY[1]

J. N. Moore[2] and E. H. Varney[3]

Abstract

Experiments were conducted to determine the influence of runner removal and time of planting in the field on the incidence of Verticillium wilt in the strawberry. Removal of runners as they emerged from the crown had no significant effect on wilt incidence when compared with plants from which runners were allowed to root. A direct proportionality existed between earliness of planting and increased incidence of wilt.

In recent years Verticillium wilt, caused by Verticillium albo-atrum Reinke & Berth., has been recognized as a serious disease of strawberries in certain areas of the eastern United States (2, 7). The introduction of new, horticulturally superior but Verticillium-susceptible varieties has contributed to an increase in the severity of the problem. Since the use of resistant varieties appears to be the most economic control for this disease, a breeding program to combine resistance to Verticillium with good horticultural characters was initiated at the New Jersey Agricultural Experiment Station in 1958 (5).

The purpose of the experiments reported here was to determine the effects of removing runners to facilitate closer plant spacing and of varying the time of planting on the development of Verticillium wilt symptoms in plants involved in the breeding program.

METHODS

These experiments were conducted in a field previously used for screening varieties and known to have a high Verticillium inoculum potential (7, 8). Each treatment was replicated four times and each replicate consisted of 25 plants planted 2 feet apart in the row.

The very susceptible variety Dixieland and the moderately susceptible variety Sparkle were included in the runner-removal study. The experiment consisted of two treatments of each variety: (a) runners removed as they emerged from the crown, and (b) runners allowed to root and form a matted row. Dormant plants were set in the field on April 28, 1960.

The time-of-planting study consisted of planting dormant, cold-stored plants of the variety Dixieland in the field at 28-day intervals beginning on April 28, 1960. A total of five plantings were made, the last on August 18, 1960. When necessary, irrigation was used immediately following planting.

Individual plants were rated for wilt on August 17 and October 4, 1960. The following scale of 0 to 5 was used: 0, no apparent wilt; 1, slight symptoms, no necrosis; 2, moderate symptoms, some necrosis; 3, extensive necrosis; 4, plant nearly dead; 5, plant dead. A disease index for each plot was obtained by totaling the individual ratings. Mean plot values were converted after analysis to a 0 to 100 scale, in which 0 indicates all plants apparently healthy, and 100 all plants dead.

RESULTS

Experiment 1 Runner Removal Study: The results of runner removal on the incidence of Verticillium are given in Table 1. The only significant difference found was the greater susceptibility of Dixieland when compared with Sparkle, regardless of runner treatment. In the case of both varieties, there was a trend toward the results obtained by Keyworth and Bennett (3), who reported a decreased severity of Verticillium in de-runnered strawberry plants. This difference was not, however, sufficiently large to be significant statistically.

Experiment 2 Time of Planting Study: The results of time of planting on the development of Verticillium wilt in the variety Dixieland are given in Table 2. The severity of the disease was found to be directly proportional to the length of time the plants had been in the field. The

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Table 1. Effect of runner removal on the incidence of Verticillium wilt in strawberries. (Ratings made October 4, 1960)

Treatment		Disease index[a]	% dead or severely wilted
Dixieland			
Runners		81.9	76.4
No runners		73.8	69.0
Sparkle			
Runners		35.7	17.4
No runners		23.6	9.2
Mod. Tukey D test	.05	26.4	30.8
	.01	35.4	40.3

[a] Based on scale in which 0 indicates all plants apparently healthy and 100 all plants dead. (See text)

Table 2. Effect of time of planting on the incidence of Verticillium wilt in strawberries. (Ratings made October 4, 1960)

Date of planting		Disease index[a]	% dead or severely wilted
April 28		80.8	79.3
May 26		57.8	52.1
June 23		30.7	24.7
July 21		12.8	7.4
August 18		0.0	0.0
Mod. Tukey Test	.05	19.3	20.5
	.01	24.9	26.5

[a] Based on scale in which 0 indicates all plants apparently healthy and 100 all plants dead. (See text)

low incidence of wilt in the July 21 and August 18 plantings may have been due to a failure of the fungus to develop at high soil temperatures as previously reported (1, 4, 6).

It should be pointed out that, although the July 21 and August 18 plantings had a low incidence of wilt, the plants did not make satisfactory growth prior to the onset of winter. The best treatment from the standpoint of strawberry production appeared to be the June 23 planting date, but for screening seedlings or selections for resistance, the April planting date was the most desirable.

CONCLUSIONS

The results obtained in these experiments indicate that plants may be de-runnered for closer plant spacing without any appreciable influence on their response to Verticillium wilt. Time of planting in the field, however, is important on the incidence of wilt. In order to get the maximum development of wilt in a seedling or variety population being screened for wilt resistance, the plants should be planted in the field as early as possible in the spring.

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NEW JERSEY AGRICULTURAL EXPERIMENT STATION, NEW BRUNSWICK

A NEW SURVIVAL RECORD FOR CERTAIN FUNGI ON AGAR UNDER A MINERAL OIL SEAL

G. L. Barnes[1]

Long-term preservation of agar cultures of bacteria, fungi, and algae under sterile mineral oil is a common practice in many laboratories when frequent transfers to fresh media are undesirable or too time consuming. With fungi, the oil seal method of preservation is generally used for the non-sporulating species which are not suitable for preservation by lyophilization. The longevity of microorganisms under mineral oil varies considerably with the species, the storage temperature, and perhaps the culture media. Many fungi have been reported to be viable after 12 to 24 months' storage under oil, but only a relatively few species have survived upwards to 14 years (3, 4, 5). A new survival record for several spore-producing, plant-pathogenic fungi is reported here.

Early in 1956, R. H. Converse[2] covered lima-bean agar slant cultures of several fungi with sterile mineral oil and stored the cotton-stoppered tubes in a refrigerator maintained between 8° and 10° C. The fungi are as follows:

Cladosporium fulvum Cke.
Cladosporium herbarum (Pers.) Lk. ex Fr.
Fusicladium carpophilum (Thuem.) Oud.
Fusicladium effusum Wint.
Monilinia fructicola (Wint.) Honey
Trichothecium roseum Lk. ex Fr.
Venturia inaequalis (Cke.) Wint.

In May 1961, the author aseptically removed small portions of mycelium from a single culture of each fungus, removed excess oil with sterile filter paper, and transferred the portions to tube slants of peptone-dextrose agar. The tubes were examined weekly for fungus growth and if growth was not evident by the end of 1 month, the mycelium was considered non-viable. Of the seven fungi, only C. fulvum and C. herbarum failed to survive the 5-year storage period under oil. The other five fungi were viable.

This is the first report on survival of F. effusum under oil. This fungus was not mentioned in the 1960 review article by Fennell (3) on conservation of fungus cultures. F. effusum is represented in the American Type Culture Collection (1) but no survival data under oil are available[3]. Cladosporium species (not specified) were previously reported to have survived up to 2 years under oil (2, 6) as has T. roseum (2), M. fructicola (7), and V. inaequalis (7). These species are represented in the American Type Culture Collection but no longevity records under oil are available from that source[3]. Fennell[4] has no additional information on these species. According to Boone[5], V. inaequalis has survived 8 to 10 years under oil when held between 4° and 8° C. Williams[6], however, states that many cultures of V. inaequalis did not survive 7 years of storage under oil (temperature not specified).

Apparently the 5 year survival of certain fungi under oil reported here is a new record for each fungus except V. inaequalis.

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[3] Personal communication (July 13, 1961) from Shuh-Wei Hwang, Curator of Mycology, American Type Culture Collection, Washington, D. C.

[4] Personal communication (July 5, 1961) from Dorothy I. Fennell, U. S. Army Quartermaster Research and Engineering Center, Natick, Massachusetts.

[5] Personal communication (October 16, 1961) from D. M. Boone, Department of Plant Pathology, University of Wisconsin, Madison.

[6] Personal communication (November 11, 1961) from E. B. Williams, Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

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SEASONAL INOCULUM DISPERSAL AND WHITE PINE STUMP INVASION BY FOMES ANNOSUS[1]

W. J. Stambaugh, F. W. Cobb, Jr., R. A. Schmidt, and F. C. Krieger[2]

Summary

Studies of the effect of microclimate on spore dispersal and subsequent stump invasion by *Fomes annosus* (Fr.) Cke. were made during monthly thinnings of 46-year-old, planted white pine (*Pinus strobus*) in Perry County, Pennsylvania from November 1960 through July 1961. Spore dispersal varied considerably but occurred during every thinning period. This variability was associated with rainfall, atmospheric humidity, wind velocity, and snow cover. The incidence of stump invasion ranged from 54% of the stumps cut in March to 96% in November and averaged 70% for the entire period.

INTRODUCTION

Fomes annosus (Fr.) Cke., a cause of root rot, often becomes established or intensified in coniferous plantings immediately following thinning operations. Deposition of air-borne spores on the freshly-cut stump surface is followed by invasion and eventual colonization of the stump and its root system with ultimate movement through root grafts or root contacts into adjacent healthy trees (1, 2, 3).

With the realization that *F. annosus* is already well distributed in Pennsylvania and that many plantations in localities where the fungus is present are in immediate need of thinning (5), the evaluation of the disease hazard expected in future thinnings became more imminent. A monthly thinning experiment was initiated to determine 1) seasonal dispersal of air-borne inoculum and its correlation with subsequent stump colonization, 2) possible existence of periods of maximal and minimal stump susceptibility to invasion, 3) the environmental factors governing this process, and 4) the rate of stump and root colonization and spread into the residual stand.

Although the study has not been completed, particularly objective 4) above, the results to date are considered of sufficient interest to be presented at this time as a preliminary report.

METHODS AND MATERIALS

Study Area: The plantation utilized in this investigation is located on the Tuscarora State Forest in the southwestern corner of Perry County, Pennsylvania. The 3.8-acre stand is composed of white pine planted in 1915 at a spacing of 5 x 5 feet. Except for a pruning operation and light thinning of suppressed trees at an early age and salvage following damage from hurricane winds in 1954, no cultural measures had been taken in the stand prior to the current thinnings.

Inoculum Availability: *F. annosus* was first detected in the plantation on March 25, 1959 when several stumps from wind-thrown trees in the salvage strip were found bearing sporophores. A complete survey was then made to determine all sporophore locations within the stand in preparation for the current study on spore dispersal. A total of 38 sporophores were found on the following substrates: stumps, 6; wind-thrown, suppressed trees, 3; stumps of wind-thrown trees (salvage strip), 14; standing dead trees (most of which had been suppressed), 14; and living trees, 1.

In addition, fruiting bodies of the fungus were present in other planted stands bordering the study area to the north and west and at scattered locations in natural Virginia pine (*Pinus virginiana*) to the east.

Spore Trapping Technique: Spore deposition within the stand was determined by utilizing the technique described by Rishbeth (4) with certain modifications as follows. As each tree was cut, one or more white pine stem sections, which served as selective spore traps for *F. annosus*, were exposed adjacent to and flush with the stump surface. The freshly-cut pine

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[1] Contribution No. 289 of the Department of Botany and Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication as paper No. 2627 in The Journal Series.

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sections measuring 1 x 7-8 cm were previously prepared in the laboratory and placed on moistened, sterilized cotton in sterile Petri dishes. After exposure in the field, the pine sections in the Petri dishes were incubated for 1 to 2 weeks at 20° C. At this time, the characteristic conidiophores of the fungus could be easily detected by examination at 60 magnifications.

White pine sections were used as spore traps to be consistent with the species in the plantation. Later tests showed white pine to be as good for spore trapping as red pine (Pinus resinosa) and Scotch pine (Pinus sylvestris).

Exposure periods of 3 to 7 hours were used during the November through March thinnings but were extended to 5 and 10 hour periods in later months to allow for the apparent decline in spore dispersal during the daylight hours. The data were transformed to number of spores deposited per hour to equate exposure times.

Pilot Study: Prior to the first thinning, a pilot study was conducted to determine 1) the selectivity of white pine stem sections for the detection of F. annosus, 2) the spore dispersal pattern within the plantation, and 3) the optimum exposure period of the pine sections to obtain reliable evidence of spore deposition. The results of the pilot study made on November 3, 1960 are summarized in Table 1. At the maximum exposure period of 4-4.5 hours (Table 1), spores were well distributed within the plantation.

Table 1. Effect of time and site of exposure of white pine sections upon detection of air-borne inoculum of F. annosus.

	Randomly placed pine sections			Pine sections near sporophores[a]		
Exposure period (hours)	total exposed	total positive	% positive	total exposed	total positive	% positive
0.5	12	3	25	7	6	86
1.0	12	2	17	6	5	84
2.5-2.8	12	5	42	5	5	100
4.0-4.5	12	8	67	3	3	100

[a] Only a limited number of colonies developed on the randomly placed sections as compared with the completely colonized sapwood area on most of the sections placed near sporophores.

Weather Records: Before the study was initiated, a weather station was established at the approximate center of the plantation. During each thinning the following records were obtained: 1) air temperatures (continuous for 1 week following each thinning) at 10 inches above ground level, or approximately stump height; 2) wind speed (continuous during the trapping period) at 40 inches above ground level determined by a standard Weather Bureau recording cup anemometer; and 3) relative humidity (intermittent during the trapping period) determined by a sling psychrometer. Depth of snow cover was measured directly at each tree felled during a particular thinning period and averaged for the plantation. Duration of rainfall was noted but the amount was not determined.

Experimental Thinning: One to seven trees in each of 96 rows were marked for cutting by the area forester. By thinning every 12th row in the plantation, or a total of 8 rows every month, approximately 25 trees were cut each month during the year.

Stump Invasion: After an incubation period of at least 1 month, each stump was sampled by the following procedure to determine stump invasion. The top cross section (0.5-1.0 inches in depth) was cut after atomizing 95% ethyl alcohol onto the cutting bar and chain of the power saw; with the section still in place on the stump, the bark was removed using a sterilized knife. The section was then enclosed and sealed in a new polyethylene bag. To prevent stump invasion following sampling, the freshly-exposed stump top was immediately treated with plastic roofing compound.

The samples were incubated at 20° C for 1 to 2 weeks and examined at 60 magnifications. The numbers and locations of invasion points, as evidenced by the development of F. annosus conidiophores, were recorded.

RESULTS

Spore Dissemination: The spore deposition of F. annosus for 3 to 10 hour periods immediately following each monthly thinning is presented in Table 2. The expected incidence of stump colonization on the basis of these results is expressed graphically in Figure 1A. The comparison of spore deposition for consecutive day and night exposure periods, each of approximately 10 hours duration, following the April through July thinning periods, is also shown in Table 2.

Table 2. Spore deposition rates of F. annosus immediately following mid-monthly thinnings of white pine.

Month	Total sections exposed	Total sections positive	Total exposure hours[a]	Total spores detected[b]	Deposition rate[c] actual	Deposition rate[c] relative	Average wind speed (mph)
			Day Exposures				
Nov. 18, 1960	52	15	150	23	.153	26.0	---
Dec. 15	54	5	223	5	.022	3.8	0.2
Jan. 12, 1961	52	18	298	38	.128	21.6	0.6
Feb. 17	50	8	237	9	.038	6.4	2.1
Mar. 14	54	1	379	1	.003	0.4	0.4
Apr. 14	53	16	208	31	.149	25.3	1.7
May 15	58	13	435	34	.078	13.3	2.1
June 15	48	12	346	19	.055	9.3	1.6
July 18	24	7	240	10	.042	7.1	1.1
			Supplementary Night Exposures[d]				
Apr. 14-15	10	10	100	35	.350	59.4	0.7
May 15-16	29	17	290	36	.124	21.1	1.0
June 15-16	24	24	324	191	.590	100.0	0.04
July 18-19	24	19	240	59	.246	41.7	0.01

[a] Total for all sections.

[b] Assumed on the basis that each colony developed on a pine section originated from a single viable spore.

[c] Actual deposition rate is the number of viable spores per section (4 in.^{2} of sapwood area/section) per hour (total spores/total exposure hours); the relative deposition rate refers to a scale of 0-100 with the maximum deposition recorded during the night of June 15-16 represented by 100.

[d] Maximum day-night exposures for the April through July thinning periods averaged 10 hours in duration.

Although spore dissemination was detected during each thinning period, extreme variation in inoculum dispersal occurred under the influence of fluctuating environmental conditions. This is exemplified by comparison of the day and night exposure periods (Table 2). In all cases, both the amounts and distributions of spore load were greater at night. This indicates that sedimentation was operating as a mechanism of deposition during periods of greatly reduced wind velocity and high atmospheric humidity.

Other apparent correlations can be made relative to the effect of microclimate upon inoculum dispersal. The lowest rate of spore deposition was recorded in March when rain and heavy mist occurred prior to and during the exposure period. It is assumed that the rain washed most of the spores from the atmosphere before trapping was begun and prevented the dispersal of any spores which may have been released during the day.

The most striking results on spore deposition were obtained during December and February when snow cover within the stand averaged 4.6 and 12.1 inches in depth, respectively. The occurrence of spore deposition at that time, though considerably reduced (Table 2), suggests spore release from sources other than basal and subterranean sporophores which were buried under a mantle of snow. During the January thinning, the uneven snow cover was such that sporophores were probably exposed in many areas, and for that reason snow was not considered inhibitory to spore release. In an effort to find additional sources of inoculum, Rishbeth's method for detecting spores on pine foliage (4, p. 253) was used. The presence of viable spores was demonstrated on the foliage of 5 of 10 trees sampled during the January thinning.

Stump Invasion: The incidence of stump invasion ranged from a minimum of 54% of the stumps cut in March to a maximum of 96% in November and averaged 70% for the period of November through May (Table 3). An apparent correlation was observed between inoculum dispersal and stump invasion (Fig. 1A).

DISCUSSION

The study of white pine invasion by F. annosus thus far indicated that the timing of thinning operations to reduce the amount of stump invasion may have no practical application in control of the disease. Although the incidence of invaded stumps varied considerably at times, no trend was established during the first 7 months of the study. However, percentage stump

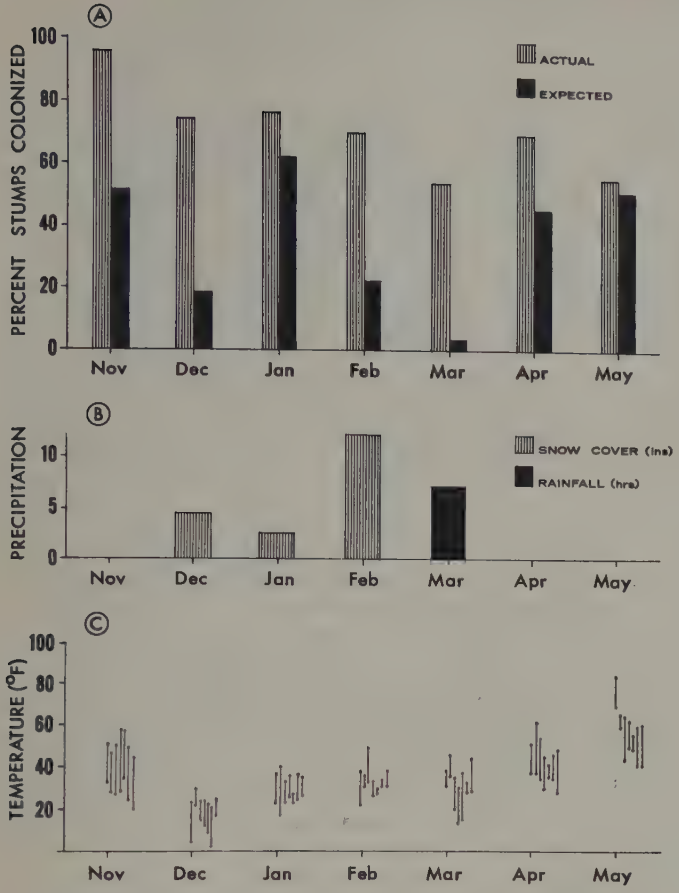

FIGURE 1. Comparison of air temperature, precipitation, and stump colonization by F. annosus. A -- Expected and actual stump colonization in relation to time of thinning. Expected values were based on the number of trap locations with spore deposition during the day of thinning. B -- Precipitation. Snow cover is expressed in inches of snow on the ground at the time of thinning and rainfall in hours' duration during the spore trapping period. C -- Maximum and minimum daily air temperatures for weekly intervals beginning on the day of thinning.

Table 3. Incidence of stump invasion and colonization by F. annosus following monthly thinning of eastern white pine.

Date of thinning	Trees cut	% stumps colonized	Invasion points total	Invasion points average per stump	Field incubation period (days)
Nov. 18, 1960	22	96	73	3.3	139-154
Dec. 15	27	71	42	1.6	112-127
Jan. 12, 1961	25	76	58	2.3	84-116
Feb. 17	23	70	38	1.6	48-97
Mar. 14	26	54	33	1.3	55-69
Apr. 14	23	70	47	1.0	35-74
May 15	27	56	36	1.3	32-43

invasion has not yet been determined for the warmer summer months. It should be pointed out that these data are based on only one cutting period per month and do not necessarily indicate the amount of invasion which might occur at other times.

Other than providing evidence of inoculum availability at the time of each thinning period, the variation in spore deposition shown by the monthly spore trapping studies does not serve as an accurate indicator of ultimate stump colonization. The length of time during which freshly-cut stumps are susceptible to invasion by F. annosus should be determined and studies on sampling the inoculum dispersal for this period should be designed accordingly. In England, Rishbeth (2) found that F. annosus can become established in stumps for a maximum period of 4 weeks and possibly longer after felling. This information should serve as a basis for conducting similar studies under Pennsylvania conditions.

In areas where inoculum is present, spore deposition can be expected to occur on all or nearly all of the stumps in a given area during any calm, 24-hour period. This is assumed on the basis of the very high spore deposition rate obtained in the supplementary night exposure studies (Table 2).

The sources of inoculum during periods of heavy snow cover have not been located. Viable spores were found on the foliage of several trees, but it is doubtful that enough inoculum can persist as foliar deposits, without replenishment from sporophores, to account for stump invasion in the magnitudes observed. Aboveground sources in addition to foliar deposits must be present. A careful search throughout the plantation and surrounding stands did not reveal any sporophores on stems above duff level. The possibility that sporophores might be present internally in heart-rotted stems at heights above the snow level and with openings for spore egress seems slight. This possibility, as well as that of conidia as a source of inoculum, needs further investigation. The complete absence of the conidial stage of _F. annosus_ in nature is difficult to envision, especially since it develops so readily on invaded pine sections kept in a moist atmosphere. Conditions suitable for conidial production in the field might be more common than heretofore supposed.

The incidence of stump invasion from November through May (Table 3) was much higher than that estimated on the basis of decay and sporophore development (10 to 30 percent incidence) in other thinned white pine stands (5). This suggests that _F. annosus_ may not survive in all invaded stumps.

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A ROOT OBSERVATION BOX AND TECHNIQUES FOR STUDYING INFECTION AND PATHOGENESIS OF ROOTS BY NEMATODES

Norman A. Minton[1]

The inoculation box (Fig. 1) is a modification of a root-observation box described by Comstock (1). The inside dimensions are 3/4 x 11 x 30 inches. The sides are constructed of 1/2-inch thick plywood and the bottom and ends of 3/4 x 3/4 inch wood strips. The box is lined inside with a 4 mil (0.004 inch) plastic film. The piece of plywood forming the back side of the box was covered with the plastic and the strips forming the bottom and ends were attached to it with 1-inch wood screws. The plastic was fastened at the top with wire staples and extended beyond the bottom of the box so it could be folded back over the front side. The removable plywood front side is attached with 1-inch wood screws. The medium in which the plants is grown is finely ground vermiculite to which a complete nutrient solution is applied.

To use the box, seedlings were germinated in quartz sand in 2-gallon crocks. When ready for use, they were floated free of the sand with a minimum of injury by submerging the crock in water. The observation box was laid flat with the front side and the plastic removed. The seedlings were equally spaced on a 1/4-inch layer of vermiculite spread in the box and arranged so that the tips of all tap roots lay on a line drawn from reference points marked on either end of the box. These points were used to relocate the inoculation sites.

Inoculum consisted of a water suspension of freshly hatched root-knot larvae collected from heavily galled tomato roots incubated in a moist chamber by the method described by Oostenbrink (2). This was distributed over a band of vermiculite 1 inch above and below the tips of the tap roots. Next, the roots were covered with a 1/2-inch layer of moist vermiculite and the plastic film and plywood cover replaced. The box was then set upright on a greenhouse bench. For infection studies, the inoculum was left around the roots, but for timed developmental and pathological studies, it was removed after the desired interval of time by washing the vermiculite from the tray with a gentle stream of water and flushing the roots thoroughly. Moist nematode-free vermiculite was again placed around the roots and the box returned to the greenhouse bench for further growth of the plants. All or any of the plants may be removed from the box at any time.

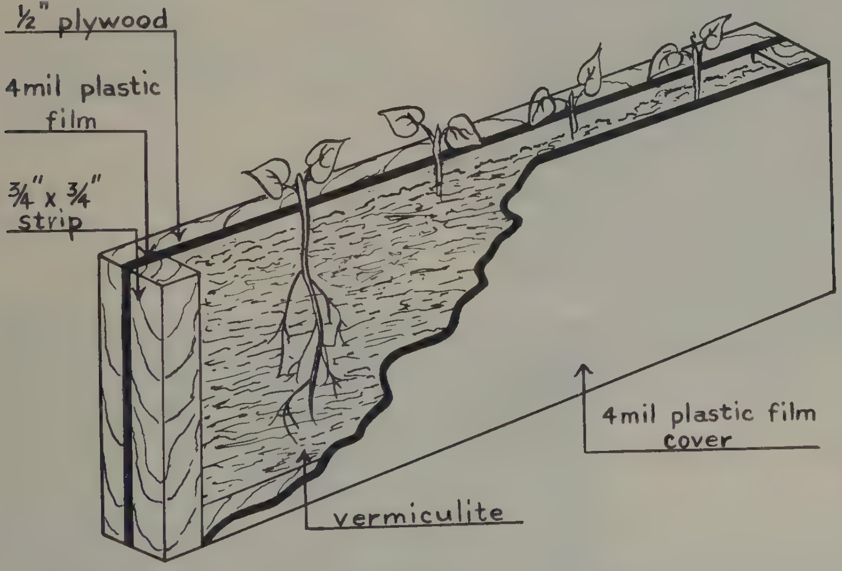

FIGURE 1. Box in which plants were inoculated with nematodes.

In investigations using this technique, nematode larvae penetrated the roots readily and the galls formed appeared to be normal. The relocation of the inoculation sites was easy and rapid. Uniform inoculation of large numbers of plants is easy and a minimum of greenhouse space is required. Vermiculite provides a standardized medium and is easily available. Nematodes other than root knot can also be studied using this technique.

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LEAF SPOT ON RANGPUR LIME AND ITS CONTROL

Julius L. Heinis[1]

Summary

Alternaria citri Ellis & Pierce was found associated with a leaf spot on Rangpur lime and related citrus species in south Texas. Occasionally *Colletotrichum gloeosporioides* Penz. was found together with *A. citri.* Captan sprayed weekly and zineb sprayed with up to a 3-week interval gave satisfactory control of the disease.

A block of 3-year-old Rangpur lime trees was grown by the Citrus Center of Texas College of Arts and Industries, Weslaco, Texas for virus indexing purposes. In May 1961 the trees were cut back to 3-foot stumps, which soon sprouted vigorously. On June 16 a leaf spot was very abundant on the young shoots, and *Alternaria citri* Ellis & Pierce was found sporulating. Most spots were oval in shape and grew to 3 x 1 cm in size (Fig. 1). Several concentric rings of tiny black dots, formed by sporophores and spores (Fig. 2) were typical. The necrotic centers were dehydrated and tore easily. The spots were surrounded by a yellow zone which gradually enlarged, finally resulting in leaf drop. Toward the end of September many shoots were defoliated.

Alternaria is known to cause a black rot on oranges and lemons (2). Fawcett (2) referred to Doidge, who in 1929 described a leaf spot on rough lemon produced by *A. citri.* Ruehle (4) in 1937 demonstrated the pathogenicity of this fungus on rough lemon in Florida. The morphology

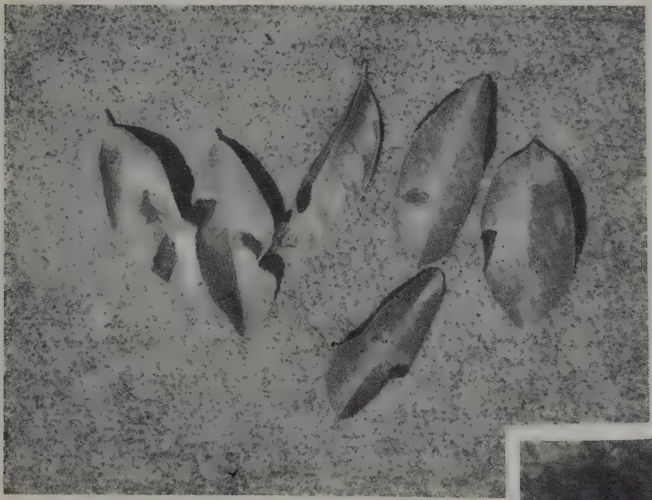

FIGURE 1. (left) Leaf spot on Rangpur lime.

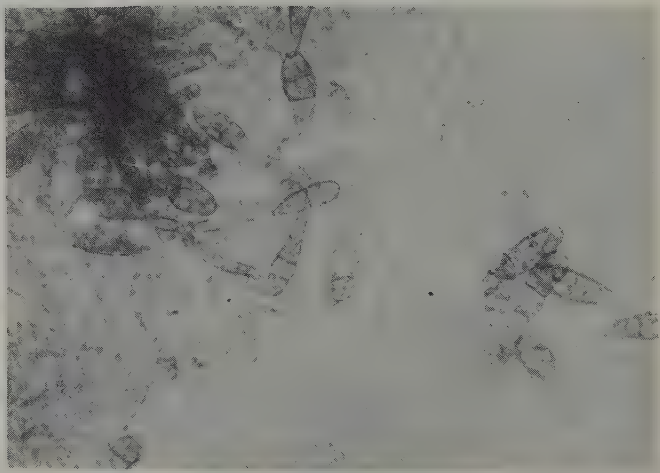

FIGURE 2. (right) *Alternaria citri* Ellis & Pierce from Rangpur lime leaf. Single spore of *Colletotrichum gloeosporioides* Penz. is seen in bottom center.

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and taxonomy of A. citri was described by Bliss and Fawcett (1) in 1944. Most authors considered Alternaria a saprophytic organism, which is widely distributed and under certain conditions attacks citrus fruits.

A leaf spot similar to the Alternaria spot is caused by Colletotrichum gloeosporioides Penz. and commonly called anthracnose. In Florida, Fisher (3) reported anthracnose as a primary problem on rough lemon and was able to control the disease with ferbam, zineb, and captan, in that order.

Other citrus species on which Alternaria leaf spot was found were Kusaie mandarin lime and rough lemon. Occasionally C. gloeosporioides was found sporulating in the same leaf spot, and on Cleopatra mandarin this fungus was predominant.

SPRAYING EQUIPMENT

A field test was run to compare the effectiveness of zineb and captan applied as sprays at intervals of 1, 2, and 3 weeks, in controlling Alternaria leaf spot on Rangpur lime. The rates used were 2 pounds of zineb per 100 gallons and 6 pounds of captan per 100 gallons of water. Plyac was added to each spray as a spreader-sticker. Ten Rangpur lime trees were used in each treatment. The first spray was made on June 23, 1961 and the last on August 21.

On September 1 the experiment was terminated and readings were taken. For sampling, the uppermost 1-year-old branch of each test tree was selected. For an estimate of the disease incidence, a count was made of all leaves having one or more leaf spots. Since the disease caused many leaves to fall, leaf scars were also counted and included in the number of diseased leaves. From this the percentage of diseased leaves was calculated with respect to the total number of leaves present and fallen.

All spray treatments were effective in reducing the incidence of Alternaria spot. Both zineb and captan gave good control when applied at weekly intervals (Table 1.) However, when individual sprayings were separated by 2 or 3 weeks, zineb was considerably better in preventing leaf spotting.

Table 1. Effect of sprays at various intervals on leaf spot and growth of Rangpur lime trees (average of 10 trees).

Treatment	Spray interval (in weeks)	% diseased leaves	Growth (in inches)
Zineb	1	4	196
	2	7	183
	3	7	144
Captan	1	4	165
	2	34	129
	3	23	142
Check	---	64	153

An attempt was made to study the effect of leaf spot on the growth of Rangpur lime by measuring the total length of the main shoot and side shoots of the samples. The results are given in Table 1. Statistical analysis showed that the growth differences between treatments were not significant. Apparently the vigor of the current year's growth on the 3-year-old test trees was not influenced by the leaf spot.

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RHYNCHOSPORIUM LEAF SCALD OF RICE IN GUATEMALA

Eugenio Schieber

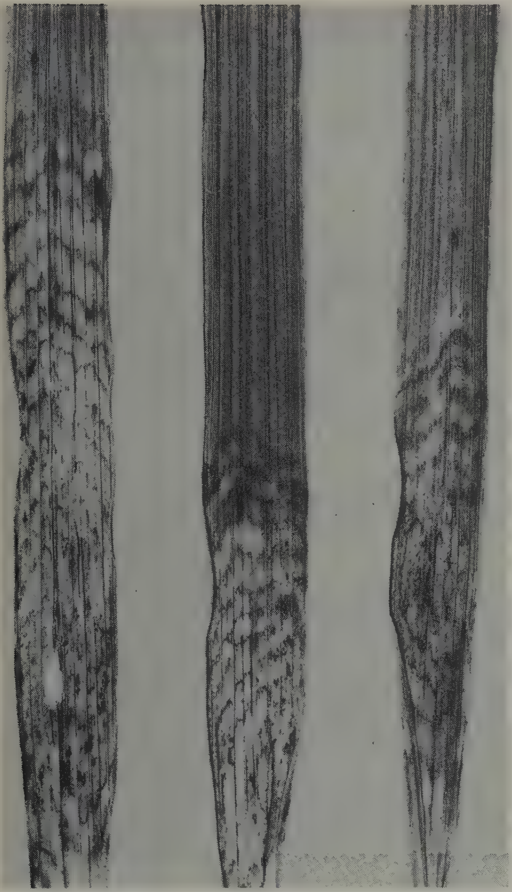

FIGURE 1. Symptoms on the leaves of Blue Bonnet, showing the typical zonate-scald, incited by R. oryzae.

Leaf scald on rice (Oryza sativa), incited by Rhynchosporium oryzae[1] Hashioka & Yokogi, was found for the first time in the Pacific Coast area of Guatemala in September 1961, attacking var. Blue Bonnet. The disease was also present in the international nurseries located at the "Cuyuta" Experiment Station of the Instituto Agropecuario Nacional. Leaf scald was also found scattered in rice plantations of local and introduced varieties along the Pacific Coast. This appears to be the first record for Guatemala and the second report for the Western Hemisphere (1).

Symptoms under conditions of natural infection were similar to the descriptions given in Japan (2) and Costa Rica (1). The symptomatology resembled also that of Rhynchosporium scald on barley incited by R. secalis (3). Seedling blight was not found as frequently as the typical scald lesions on the leaves. Lesions developed mainly from the tips of the leaves. The initial yellowing progressed basally and the older lesion was straw-colored with brown transverse zonations (Fig. 1). The lesions occur less frequently lower on the leaf blade and extend into the leaf-sheath. The zonate-scald area constitutes the typical symptom. Occasionally the diseased mesophyll tissues break down giving a shot-hole effect to the lesion.

Conidia are hyaline, one-septate, cylindrical, slightly curved, and without the short apical beak typical of R. secalis (3).

Previous workers (1, 2) reported that the disease was more prevalent in rice grown under high nitrogen fertilization. At the "Cuyuta" Experiment Station, the disease was uniformly distributed on plants in all plots in a fertilizer trial conducted during 1961 when leaf scald was observed for the first time.

The reaction of varieties in the nurseries to the disease was similar although the variety "Dima," a recent introduction from Surinam and highly resistant to the hoja blanca disease of rice, and "Gulfrose," a recent introduction from the United States, were only slightly attacked by R. oryzae.

Workers in Japan and Costa Rica reported that Rhynchosporium leaf scald on rice was increasing in severity in recent years. On this basis the disease may become of importance in Guatemala.

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[1] Thanks are due to Dr. Eddie Echandi, IICA Turrialba, Costa Rica for help in the identification.

FUSARIUM ROOT ROT OF MAIZE IN ISRAEL

A. Joffe[1]

In late summer of 1961, maize sown under overhead irrigation on heavy soil in various parts of the Israel coastal plain was affected by a rot of roots and rootlets. Plants showed symptoms only 6 weeks or more after sowing, and then dried up progressively from the lowest to the higher leaves.

Cultures from affected roots were made on 10 selective substrates. The causal organism was determined to be *Fusarium moniliforme* Sheldon.

This diagnosis agrees with Gordon's (2) identification of *F. moniliforme* in cultures isolated from roots of maize and sorghum in Israel. The same fungus has been isolated by us from soil of a rotation experiment, carried out in the central coastal plain, in which maize is being grown for almost 40 years in a 5-year rotation of unirrigated crops (3). But in this case no disease was observed on the maize.

The fact that in Israel the seedling blight commonly caused by this species of *Fusarium* in other countries (1) has not been observed, may be due to the dry summer climate of Israel. In the absence of rain, the soil around seedlings spaced singly or in pairs dries up rapidly after each irrigation. The disease only seems to develop when dense growth of older plants shades the soil continuously and keeps it moist for extended periods.

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POWDERY MILDEW OF SAFFLOWER

Arthur H. McCain

Powdery mildew of safflower, *Carthamus tinctorius*, caused by *Leveillula taurica* f. sp. *carthami* has been reported from Europe (5), Asia (4, 8) and the Sudan (7). Powdery mildew of safflower caused by undetermined *Oidium* species has been reported from Siberia (6), France (3), Israel (1) and recently from Arizona on greenhouse grown safflower (2). Darpoux (3) stated that the *Oidium* severe on safflower in France was probably *Erysiphe cichoracearum* f. sp. *carthami*. An *Erysiphe* species, probably *E. cichoracearum*, has been found on greenhouse-grown safflower seedlings in Utah (9).

In the fall and winter of 1959 powdery mildew was observed on mature safflower leaves in a greenhouse maintained between 45° and 70° F, but not in warmer sections maintained between 70° and 80°. Cross inoculation studies revealed that the fungus was the same one causing powdery mildew on cucumber, *Cucumis sativus*. The fungus is presumably *Erysiphe cichoracearum* DC.

Powdery mildew has not been observed on field grown safflower plants in California.

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BOOK REVIEWS

"THE GENUS TILLETIA," by Ruben Duran and George W. Fischer, Washington State University, Pullman, Washington. 138 pages. 64 text figures. 1961. Price $2.75.

The genus Tilletia includes some of the most important pathogens of the cereal and forage crop plants of the world. In fact all of the species are parasitic in behavior and with two exceptions occur on grasses. The annual loss from these ubiquitous organisms still reaches a staggering total despite the efforts of phytopathologists through the years. The literature on the taxonomy, morphology, physiology, genetics, and pathogenicity is voluminous and widely scattered, often in obscure journals and out of the way places. As is so often the case with other large and complex fungus genera, many of the species have been inadequately described or named without reference to earlier names for the same form. This situation points up the need for a critical review of the genus and a systematic presentation of the species involved.

This the authors have accomplished in the present book. They present an historical review and outline the methods and materials used involving not only their own collections, but specimens loaned from more than 60 mycological herbaria of the world. Morphological characters to be used as criteria in establishing good specific concepts are limited in the smut fungi and the authors discuss concisely those available to them, including position and form of the sorus, color of the spore mass, and ornamentation, color, and size of the spores.

The descriptive section is prefaced by a redrawn description of the genus, an analytical key and a host - genus key to the species. To the 73 older species accepted, the authors, happily, have found it necessary to add only three, for only one of which they are directly responsible. The remainder of the more than 200 binomials to be found in the literature revert to synonymy or to the section for doubtful and excluded species. Spores of 64 of the species are adequately illustrated. All specific descriptions have been redrawn following a uniform plan.

This book will prove exceedingly useful to all mycologists, plant pathologists and others concerned with the genus Tilletia. - - JOHN A. STEVENSON

"FUNGICIDES IN AGRICULTURE AND HORTICULTURE," S. C. I. Monograph No. 15. Staples Printers Limited, Rochester, Kent, England. iv + 145 pages. 1961. Price £1. 10. 0.

This monograph is composed of a group of papers read in four sessions of a Symposium organized by the Committee of the Pesticides Group of the Society of Chemical Industry, held at the London School of Hygiene and Tropical Medicine on March 20-21, 1961.

The purpose of the Symposium, in the words of the Chairman, G. L. Baldit, was the joint discussion by British and Dutch chemists and plant pathologists of the present and future place of fungicides in agriculture and horticulture. Eleven papers were presented, with a discussion period after each.

Although the use of fungicides dates to the discovery of Bordeaux mixture in 1885, the development in 1931 of the first effective organic fungicide revolutionized the approach to research and paved the way to a new era in plant disease control. Hence, it is on these last 30 years of organic fungicide research that stress is laid in this Symposium, with emphasis on the most important group, the dithiocarbamates.

Topics covered include usage of modern fungicides, mode of action of chemicals on fungi, new developments in organics, natural host resistance to disease, effects of both old and new materials against specific diseases, and here and there a backward look as well as a prognosis of the future.

In the first session on usage the relative merits of the more effective of the generic- or common-name organics are compared. The first paper deals with control of diseases of soft, stone, and pome fruits; the second with fungicides and fumigants used in England's glasshouse crops; and the third with fungicide usage in world agriculture.

The second and third sessions, which are highly technical and largely chemical in nature, are devoted to such esoteric subjects as measurement of the in vitro fungistatic action of chemicals on spores, the manner in which accumulated fungicide reacts on the fungal cell and what controls the accumulation process; and the relation between critical receptor sites and lipoid solubility in the structure-activity area that can prove toxic to fungal tissue.

The first paper in the fourth session is concerned with a study of the natural chemical protection afforded the broad bean plant by antifungal compounds manufactured by the plant itself, and the isolation and analysis of the chemical property in an effort to explain its role in natural disease resistance. The two final papers are on application. The first introduces a new fungicide developed by a British firm that shows promise for control of Botrytis on lettuce and flower crops. The second, on the well-known narrow-spectrum fungicide dodine, suggests a recommended spray schedule for apple scab control.

Several conclusions emerge from the Symposium. Great strides have already been made in fungicide research, but still greater progress could be made in the future. The organics will undoubtedly continue to dominate the fungicide field, where there is still need for chemicals that will control soil-inhabiting fungi, act as curatives rather than as protectants, and have an improved cost-benefit ratio for control of such diseases as wheat rust.

Because of its limited scope, this little book probably will have only a narrow reader interest. It will be of little value to the novice, or even to the seasoned plant pathologist interested primarily in application and results. But it is most heartily recommended to those scientists who are actively engaged in fungicide development and to others who maintain a lively interest in this burgeoning new aspect of our science. -- HILDE McGRATH

ANNOUNCEMENT

Since his retirement, Dr. C. D. Sherbakoff, Department of Plant Pathology, University of Tennessee, Knoxville, has been reviewing his interest in studies on Fusaria. He would like to obtain a large number of specimens of Irish potato rot, from as many States as possible, which he hopes will contain Fusaria. These specimens need not be bulky; a 1/4-inch slice of the tuber, which has been partially air dried, would provide Dr. Sherbakoff with sufficient material from which to make isolations.

THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 46 Number 4

April 15, 1962

Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

23 MAY 1962

SUGGESTIONS FOR PREPARATION OF MANUSCRIPTS

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

(2) MANUSCRIPTS should be the original ribbon copy, not carbons, clearly typed and double-spaced throughout, including tables, footnotes, and literature cited. Submission implies nonsubmission elsewhere and (if accepted) no publication elsewhere in the same form without consent.

(3) ABSTRACTS are requested for all except very short articles.

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so.

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Paul R. Miller

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CONTENTS

1. Tatter leaf, a previously undescribed virus effect on citrus
J. M. WALLACE and R. J. DRAKE 211

2. Inhibition of sugarcane mosaic virus by milk
LOUIS ANZALONE, Jr. 213

3. An evaluation of sodium hypochlorite soil applications for control of Dutch elm disease
A. H. EPSTEIN and E. B. SMALLEY 216

4. The perfect stage of Sphaerotheca macularis on strawberry plants
J. S. JHOOTY and W. E. McKEEN 218

5. Kernel-spot of filberts found in Oregon
ROBERT S. HALLIWELL and P. W. MILLER 220

6. Reduction of peach decays by hydrocooling with chemical solutions and chemically treated ice
W. L. SMITH, Jr. and W. H. REDIT 221

7. An evaluation of two fungicides as soil treatments with and without herbicide for tomato fruit disease control
D. F. CROSSAN and A. L. MOREHART 227

8. Dr. Thomas Taylor and the delinting of cotton seed
JOHN A. STEVENSON 230

9. Control of pecan scab in Alabama in 1961 and its relation to nut quality
URBAN L. DIENER 232

10. An evaluation of certain fungicide - adjuvant combinations for control of pecan scab
GEORGE L. BARNES 236

11. Systemic insecticides for indirect control of bean yellow mosaic virus in seed fields of yellow lupine
D. B. LEUCK, et al. 240

12. Comparison of flowering crab apple varieties for fast detection of a common latent virus in apples
J. E. REYNOLDS and J. A. MILBRATH 243

13. Chloropicrin injury on sweetpotatoes
R. A. CAPPELLINI and R. H. DAINES 246

14. A second pathogenic race of Pseudomonas sesami
C. A. THOMAS, et al. 248

15. Control of bacterial spot of peach with dodine
ROBERT H. DAINES 251

16. Sprout dip treatments for control of black rot of sweetpotatoes
ROBERT H. DAINES 253

17. Effect of herbicides on nematodes in bentgrass
WILBUR D. COURTNEY, et al. 256

18. Effects of fungicidal drenches on root infestation by Rhizoctonia solani and incidence of two-spotted spider mites on strawberries
E. M. STODDARD and P. M. MILLER 258

19. An unusual local epidemic of tomato ghost spot
R. E. WEBB 260

20. Botryosphaeria twig blight of Arizona cypress
E. S. LUTTRELL, et al. 261

21. Association of Cladosporium with needle cast of larch and spruce in the United States
RICHARD CAMPANA and MARTIN ROSINSKI 265

22. Incompatibility of Price and Cook Nucellar Eureka lemon trees on No. 1452 citrumelo rootstock
J. D. KIRKPATRICK, et al. 267

23. A chlorotic decline of ponderosa pine in southern California
J. R. PARMETER, et al. 269

24. Variation in virulence of Valsa leucostoma
DAVID S. WYSONG and LESTER E. DICKENS 274

25. The comparative resistance of several inbred corn lines to bacterial wilt and leaf blight
J. L. PETERSON and J. C. ANDERSON 277

26. Zonate ringspot, a new virus disease of bleeding-heart, Clerodendrum thomsoniae
HARRY C. BURNETT and CHARLES O. YOUTSEY 279

27. Nematode populations and the growth of apple trees
S. E. TAMBURO and ROBERT E. ADAMS 281

28. A basal canker disease of eastern white pine
S. B. SILVERBORG, et al. 285

29. Weak peach, a new virus disease of peach
GLENN KenKNIGHT 287

30. Studies on some graminicolous species of Helminthosporium stored under oil
J. L. PETERSON and R. A. CAPPELLINI 290

31. Leafhopper vectors of Pierce's disease virus in Georgia
GEORGE H. KALOOSTIAN, et al. 292

32. Medlar is host for Gymnosporangium clavipes
JERRY T. WALKER and ELLEN F. EARHART 293

33. Diseases of rice new to El Salvador
OSCAR ANCALMO and WILLIAM C. DAVIS 293

34. Book Review (How to Control Plant Diseases in Home and Garden)
PAUL R. MILLER 294

35. Results of 1961 fungicide and nematocide tests 294

TATTER LEAF, A PREVIOUSLY UNDESCRIBED VIRUS EFFECT ON CITRUS

J. M. Wallace and R. J. Drake[1]

Summary

Seedling plants of Mexican lime, Citrus aurantifolia, and Kalpi lime, C. excelsa, displayed a blotchy spotting on young leaves following inoculation from a Meyer lemon that was known to be infected with the tristeza-seedling yellows virus complex. As the leaves of C. excelsa enlarged they were irregular in shape with uneven or ragged margins. Experimental study indicated that the tristeza-seedling yellows virus complex was not the cause of these symptoms. Although the spotting of the young, soft leaves resembled symptoms of psorosis it was not possible to attribute either the young leaf symptoms or the distorted mature leaves to psorosis. For purposes of description and identification this apparently new citrus virus disease has been given the name "tatter leaf."

The original Meyer dwarf lemon trees established in the United States were brought from China in 1908. Since then many thousands of vegetatively propagated trees, cuttings or budlings, have been grown from this introduction for use as dooryard and ornamental planting. It has been established that the small trees that came from China were carriers of virus that causes the tristeza reaction (vein-clearing and stem pitting) on seedlings of Mexican lime (1, 3, 4, 5, 6). Other studies in California (unpublished) proved that inoculations from Meyer lemon plants resulted in typical tristeza on budded trees of sweet orange, C. sinensis, on sour orange, C. aurantium, rootstock. With few exceptions all Meyer lemon plants in California and Arizona that have been tested have proved to be carriers of this virus. The exceptions are trees that have been grown either from a seedling of Meyer lemon or possibly from buds which by chance were virus-free when they were removed from the parent tree.

Subsequent investigations (7, 9) have demonstrated that all Meyer lemon trees in California and Arizona that are infected with virus that induces the lime reaction and which have been indexed on Eureka lemon are also carriers of a second virus that causes the seedling yellows reaction on lemon, grapefruit and sour orange, originally described by Fraser in Australia (2). Investigations in both Australia and California have disclosed that there are either two distinct viruses or two related virus strains in plants that permanently carry virus that will cause symptoms of seedling yellows. Until the relationship of these components is established the writers choose to refer to the mixture as "tristeza-seedling yellows virus complex."

Trees of Meyer lemon propagated from the original introductions from China were planted at the Citrus Research Center in Riverside, California in 1917. One of these and a daughter tree propagated from it in 1930, both infected with the tristeza-seedling yellows virus, appear to be carriers of another virus not previously described. This virus causes a blotchy, spotting of young leaves of Mexican lime, Citrus aurantifolia, and Kalpi lime, C. excelsa. Also, the leaves of Kalpi lime are reduced in size, abnormal in shape and have irregular, somewhat ragged margins. Because of this latter effect the name "tatter leaf" has been given to this disease. Symptoms on leaves of Kalpi lime are shown in Figure 1.

Experimental inoculations have established that none of the known strains of psorosis, tristeza, vein enation or seedling yellows viruses cause the symptoms of tatter leaf. Cross-protection tests (8) showed that the tatter-leaf virus is not related to psorosis virus. Further, it has been established that mixtures of tristeza and different psorosis viruses do not induce the tatter-leaf symptoms.

If tatter-leaf virus is present at all in other citrus in California it has not been discovered. In a current citrus variety improvement program in this State, selected trees of commercially-used varieties are being indexed to establish sources of virus-free budwood. C. excelsa is one of several species and varieties being used as virus indicators in this program. To date more than 125 field trees of many different species and varieties have been indexed. Tatter-leaf virus has not been discovered except in three Meyer lemon plants. Additionally, 150 foreign citrus imports have been indexed on C. excelsa during the past 6 years but no tatter-leaf virus has been disclosed in this material.

---

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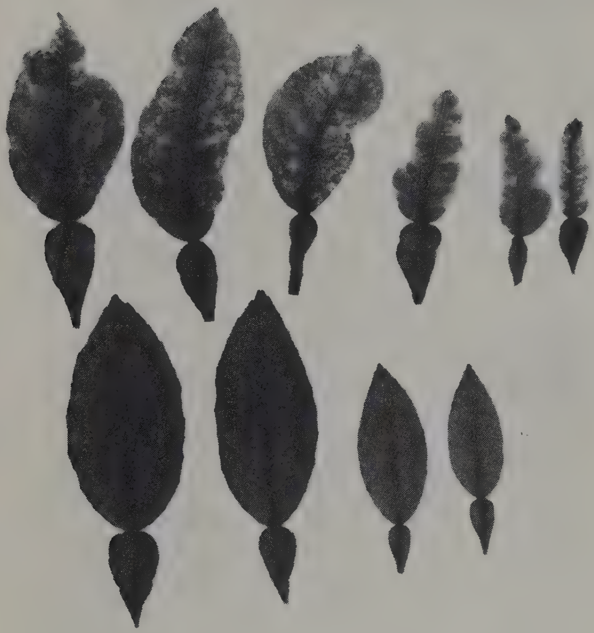

FIGURE 1. (Upper) Leaves of Kalpi lime with mottling and irregular margins of "tatter leaf." (Lower) Normal leaves.

The second case of tatter-leaf virus arose in some inoculations from a Meyer lemon tree that was not infected with the tristeza-seedling yellows virus complex. Repeated indexing of this tree resulted in psorosis-like leaf spotting on Mexican lime but no psorosis symptoms on other indicators of psorosis. Inoculations from this source to plants of C. excelsa produced symptoms of tatter leaf. A third Meyer lemon source from which tatter-leaf virus was obtained was introduced into California from Texas in 1958. Budwood from a normal, vigorous tree growing on sour orange rootstock was suspected of being free of tristeza-seedling yellows. It was introduced into the California variety improvement program and indexed for virus infection under the usual quarantine conditions practiced in this program. When the several kinds of virus indicator seedlings were inoculated from this Meyer lemon, plants of C. excelsa developed characteristic symptoms of tatter leaf[2].

Although the evidence is good that the tatter-leaf symptoms are not caused by viruses of psorosis, tristeza, seedling yellows or vein enation, the possibility remains that they may be caused by one of the recognized citrus viruses for which no leaf symptoms are known. However, from preliminary studies it appears that tatter leaf is not caused by any of the viruses of citrus diseases previously identified in the United States. Studies are now being initiated to learn if all of the vegetatively propagated descendants of the original Meyer lemon importation are carriers of tatter-leaf virus. If this proves to be so it will demonstrate that, in addition to tristeza-seedling yellows mixture, this citrus introduction from the Orient carried a third virus.

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[2] C. N. Roistacher, University of California, conducted the indexing tests on this material and discovered that it carried the tatter-leaf virus.

INHIBITION OF SUGARCANE MOSAIC VIRUS BY MILK

Louis Anzalone, Jr.

Abstract

Infection by sugarcane mosaic virus was greatly inhibited when juice from mosaic-infected sugarcane plants was either mixed at various dilutions with milk and inoculated into susceptible plants of sugarcane and sorghum, or when milk was used as a protective spray on these plants 24 hours before inoculation. In general, inhibition of infection by the virus by milk sprays decreased as the ratio of water to milk increased. Whole milk sprays, when applied to young sugarcane plants in the greenhouse in mid-summer, were phytotoxic.

Sugarcane mosaic, considered one of the most destructive diseases of the crop, is at the present time controlled almost exclusively by the use of resistant varieties. However, since new strains of the virus have been shown to cause serious damage to some commercially important varieties, studies were conducted in an effort to obtain possible control of the disease by the use of anti-viral agents.

A number of materials were screened on young sugarcane and sorghum plants in the greenhouse to determine their possible inhibitory effects on the sugarcane mosaic virus. Among these, whole milk was tested in two series of tests as an inhibiting agent against the development of mosaic symptoms.

Milk has been reported to be an effective inhibitor of plant viruses (2, 6). Johnson (6), using a modified agar-disc method, found that cow's milk was a very effective inhibitor of tobacco mosaic virus 1. Hare and Lucas (4, 5, 8), in 1959 and 1960, reported that milk inhibited the tobacco mosaic virus when used as a protective spray or when the virus was brought in contact with milk and then applied to susceptible plants. Later, Lucas (7) reported inhibition of alfalfa and cucumber mosaics, potato X and Y, tobacco etch, tobacco mosaic, and tobacco ringspot viruses with milk.

MATERIALS AND METHODS

C. P. 52-68, a commercial variety of sugarcane, and Tracy, a sorghum variety, were used as test plants in all experiments. Expressed juice of the sugarcane variety C. P. 44-101 infected with strain H of the mosaic virus served as inoculum, and grade A, homogenized and pasteurized milk served as an inhibitor. Test plants were inoculated by the air-blast (3), or sand-rub (1) methods. The air-blast method was used with or without an abrasive (Clemtex sugar sand No. 5) at an air pressure of 125 to 150 pounds per square inch. The sand-rub method consisted of rubbing the leaves of a seedling by hand with inoculum containing either river sand or sugar sand No. 5. Sugarcane plants were inoculated 13 to 19 days after planting and symptom readings were taken approximately 37 days later. Sorghum plants were inoculated when 5 to 13 days old and symptom readings were taken approximately 10 to 20 days later. All tests were conducted under greenhouse conditions. All data are given as the % of treated plants infected with sugarcane mosaic virus.

RESULTS

One test series was designed to determine the effectiveness of whole milk in inhibiting the activity of the mosaic virus when mixed with infective cane juice. In this group, milk was mixed at various ratios with mosaic-diseased juice and either rubbed or air-blasted onto young plants. Diseased juice was mixed with water to inoculate check plants. The results of tests are given in Tables 1 and 2.

These data show that infection by the sugarcane mosaic virus was completely inhibited by milk in tests when juices from virus-infected plants were inoculated into healthy plants. This occurred with juice-milk ratios of 1:2, 1:1, 2:1, and 4:1.

In another test series, various dilutions of milk and distilled water were applied as protective sprays on young susceptible sugarcane and sorghum plants. In these tests, the various dilutions of milk were sprayed on the test plants, allowed to dry for 24 hours, and the plants were then inoculated with infective sugarcane juice. Results from these tests are given in

Table 1. Effects of milk as an inhibiting agent on the development of mosaic on sorghum plants. The air-blast method without an abrasive was used to inoculate the plant.

Dilution ratio	No. of plants treated	% of plants infected
Parts of Infective Juice (I. J.) : Part of Milk or Water		
1 (I. J.) : 1 Water	777	32.2
1 (I. J.) : 1 Milk	778	0.0
2 (I. J.) : 1 Water	663	40.6
2 (I. J.) : 1 Water	685	0.0
4 (I. J.) : 1 Water	454	68.8
4 (I. J.) : 1 Milk	478	0.0
Nontreated check	741	0.0

Table 2. Effect of milk as an inhibiting agent on the development of mosaic in sugarcane (C. P. 52-68) and sorghum (Tracy) plants. An abrasive (sugar sand No. 5) was used in the inoculum.

	Air-blast method				Sand-rub method				Total	
	Cane		Sorghum		Cane		Sorghum			
Dilution ratio	no. plants	% infec.	no. plants	% infec.	no. plants	% infec.	no. plants	% infec.	no. plants	% infec.
Parts of Infective Juice (I. J.) : Parts of Milk or Water										
1 (I. J.) : 1 Water	12	16.7	40	17.5	12	25.0	41	0.0	105	11.4
1 (I. J.) : 1 Milk	12	0.0	44	0.0	12	0.0	42	0.0	110	0.0
2 (I. J.) : 1 Water	12	41.7	43	14.0	12	8.3	39	2.6	106	12.3
2 (I. J.) : 1 Milk	12	0.0	35	0.0	12	0.0	45	0.0	104	0.0
1 (I. J.) : 2 Water	12	25.0	37	5.4	12	0.0	39	0.0	100	5.0
1 (I. J.) : 2 Milk	12	0.0	37	0.0	12	0.0	41	0.0	102	0.0
Nontreated check	12	0.0	44	0.0	12	0.0	49	0.0	117	0.0

Table 3. Effect of milk used as a protective spray on the development of mosaic on C. P. 52-68. The sand-rub method with an abrasive (river sand) was used to inoculate the plants.

	No. of plants treated	% infection
Sprayed with distilled water; inoculated after 24 hours	16	43.7
Sprayed with whole milk; inoculated after 24 hours	16	0.0
Sprayed with distilled water; no inoculation	9	0.0

Table 4. Effect of milk used as a protective spray on the development of mosaic on susceptible sugarcane and sorghum plants. The air-blast method with an abrasive (sugar sand No. 5) was used to inoculate the plant.

Spray treatment ratio	Sugarcane		Sorghum		Total	
Water : Milk	no. plants	% infec.	no. plants	% infec.	no. plants	% infec.
Inoculated						
Distilled Water	12	50.0	40	35.0	52	38.5
Whole Milk	12	0.0	39	2.6	51	2.0
1 : 1	12	25.0	34	0.0	46	6.5
2 : 1	12	8.3	40	5.0	52	5.8
4 : 1	12	41.7	38	0.0	50	10.0
8 : 1	12	25.0	41	7.3	53	11.3
Noninoculated						
Distilled Water	12	0.0	41	0.0	53	0.0

Table 5. Effect of milk used as a protective spray on the development of sugarcane mosaic on sorghum plants. Air-blast method with no abrasive was used to inoculate the plants.

Spray treatment ratio Water : Milk	No. plants	% infection
Inoculated		
Distilled Water	360	35.80
Whole Milk	294	0.00
1 : 1	398	0.25
2 : 1	369	0.00
4 : 1	404	0.25
8 : 1	414	0.72
Noninoculated		
Nontreated check	399	0.00

Tables 3, 4, and 5.

The development of sugarcane mosaic symptoms was greatly inhibited in all tests when susceptible plants were first sprayed with whole milk and inoculated with the mosaic virus 24 hours later. In general the degree of inhibition decreased as the ratio of water to milk increased.

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AN EVALUATION OF SODIUM HYPOCHLORITE SOIL APPLICATIONS FOR CONTROL OF DUTCH ELM DISEASE

A. H. Epstein[1] and E. B. Smalley[2]

Abstract

Therapeutic and protectant treatments with sodium hypochlorite solutions applied as a soil drench to potted American elm seedlings at concentrations from 1 to 5000 ppm (expressed as active chlorine) were not effective in controlling Dutch elm disease. Concentrations of 1000 ppm or higher were highly toxic to the elms.

INTRODUCTION

Since its discovery, Dutch elm disease has received ample attention from the press, and with considerable justification. The disease today threatens the American elm, Ulmus americana, with extinction. By virtue of this publicity the name "Dutch Elm Disease" has become so well known to the general public that on more than one occasion the disease has even been the subject of magazine cartoons.

It should not be unexpected, therefore, that through the years the disease has had more than its share of quick and easy "cures." Often these claims have had the sanction of the United States Patent Office. These claims have seldom been supported by reliable data, and when tested under controlled conditions have usually proved worthless. For example, Neely and Himelick[3] recently demonstrated that "Soil Life" (active ingredients not defined) and "Benox" (active ingredient-oxyquinoline benzoate) had no value in the control of Dutch elm disease. However, claims of cure often remain untested or, if tested, unreported because of poor control.

Recent claims about the merits of sodium hypochlorite solutions applied as a soil drench for Dutch elm disease control have attracted widespread newspaper publicity in the Midwest. As a result, a number of communities and individuals are considering a relaxation of their normal, effective Dutch elm disease control activities in favor of the sodium hypochlorite treatments.

Because sodium hypochlorite solutions are in wide commercial use as biocides, these studies were initiated to determine their protective and therapeutic value as soil drenches against Dutch elm disease.

MATERIALS AND METHODS

The American elm seedlings used in the tests were started from 1-year-old dormant nursery seedlings and grown in 4-inch pots in a temperature controlled (65° F night - 75° day) greenhouse under incandescent illumination (16 hour day).

Solutions for treatment were prepared from commercial Klenzade (6.40% sodium hypochlorite) diluted according to the manufacturer's directions to obtain 0, 1, 10, 100, 1000, and 5000 ppm of active chlorine. Ten plants were treated at each concentration by wetting the soil with the test solutions to field capacity. Plants in the therapy series were inoculated 7 days prior to treatment, while those in the protectant series were inoculated 1 day after treatment.

Plants were inoculated with a spore suspension of Ceratocystis ulmi (Buis.) C. Moreau (approx. 100,000 spores/ml) introduced into a 1 x 3 mm bore hole in the stem 10 cm above the soil line which was then covered with masking tape.

Thirty days after inoculation, the effects of treatments were rated according to the extent of fungal colonization of the vascular system as indicated by discoloration and foliar symptoms.

RESULTS AND DISCUSSION

The solution containing high concentrations of chlorine (1000 ppm and 5000 ppm) caused radical soil changes. Soil color after treatment changed from dark brown to a pale yellow brown. There was also a noticeable alteration in the soil structure (puddling) which materially reduced the rate of water penetration.

---

[1] Plant Pathologist, Wisconsin State Department of Agriculture, Madison.

[2] Assistant Professor of Plant Pathology, University of Wisconsin, Madison.

[3] Neely, D., and E. B. Himelick. 1962. Dutch elm disease and oak wilt "cures." Arborist's News 27(1): 1-3.

Table 1. Effect of protectant[a] soil applications of sodium hypochlorite solutions upon the extent of vascular discoloration and foliar symptoms on greenhouse grown elms 30 days after inoculation.

Chlorine concentration (ppm)	No. of plants	Chemical toxicity	Plants totally invaded by *C. ulmi*	Average length of discoloration (cm)	Plants expressing foliar symptoms		
					Yellows	Wilt	Total
0	10	None	10	42.3	9	3	10
1	10	None	8	41.0	6	2	8
10	10	None	8	37.0	1	6	7
100	10	None	8	31.9	3	5	7
1000	10	Complete to partial defoliation	9	38.9	Not readable		
5000	10	Dead	--	--	Not readable		

[a] Plants inoculated 1 day after treatment.

Table 2. Effect of therapeutant[a] soil applications of sodium hypochlorite solutions upon the extent of vascular discoloration and foliar symptoms developing on greenhouse grown elms 30 days after inoculation.

Chlorine concentration (ppm)	No. of plants	Chemical toxicity	Plants totally invaded by *C. ulmi*	Average length of discoloration (cm)	Plants expressing foliar symptoms		
					Yellows	Wilt	Total
0	10	None	7	30.3	2	7	7
1	10	None	7	31.9	4	2	6
10	10	None	8	30.5	5	1	6
100	10	None	9	40.1	4	5	9
1000	10	Partial to complete defoliation	8	30.5	Not readable		
5000	10	Dead	--	--	Not readable		

[a] Plants inoculated 7 days prior to treatment.

Phytotoxicity was apparent within 24 hours at the 5000 ppm level. By the fourth day the leaves of the seedlings treated at this concentration were flaccid and water-soaked, and after 10 days all the plants were dead. Plants treated with 1000 ppm of active chlorine displayed extensive marginal and interveinal necrosis and many of the plants were defoliated. No phytotoxicity was observed at the concentrations lower than 1000 ppm (Tables 1, 2).

No significant control of Dutch elm disease was noted in any of the treatments, either protectant or therapeutic (Tables 1, 2). It is interesting to note that at the 1000 ppm concentration, in spite of the fact that the plants were defoliated by chemical toxicity, the fungus still was able to colonize completely the plant's vascular system.

Sodium hypochlorite solutions have no value either as protectants or as therapeutants against the Dutch elm disease.

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THE PERFECT STAGE OF SPHAEROTHECA MACULARIS ON STRAWBERRY PLANTS[1]

J. S. Jhooty and W. E. McKeen[2]

The perfect stage of Sphaerotheca macularis (Wallr. ex Fr.) Cooke on strawberry plants has not been described in North America. The first observation of the perfect stage of S. macularis on strawberry plants in Canada was made in the autumn of 1959 on some Fragaria chiloensis plants growing in the greenhouse. In England, during 1960, Khan (2) reported the first occurrence of the sexual stage of Sphaerotheca humuli (S. macularis) on strawberry plants growing in an unheated greenhouse. He found that the perithecia were formed in early July and occurred only on the stolons and pedicels of individual fruits, but not on the leaves.

Various species and varieties of strawberries grown for research purposes in greenhouses in London, Canada were heavily infected with powdery mildew, especially during the warm season of the year. In October, before environmental conditions for development of asexual conidia became less favourable, perithecia of S. macularis appeared on the lower surface of some infected leaves, but not on the upper surface where mildew was also abundant. The perfect stage was never observed on petioles or stolons.

The sexual bodies are white to transparent at first, but as development continues they change to light tan to brown and finally become black. White mycelium of the fungus forms a meshwork over the perithecia (Fig. 1). The spherical perithecia are 75 to 95μ in diameter and have a thick leathery covering and long unbranched simple appendages (Fig. 2). The perithecium contains one ascus which is released through its characteristic wedge-shaped rupture (Fig. 3). The ascus is elliptical, double-walled except for a single-walled thin apical zone (Fig. 4), and measures 50 to 60μ by 55 to 80μ. The ascopores are released through a split near the apical zone of the ascus (Fig. 5). Usually each ascus contains eight ascospores, but asci with fewer ascospores are also found. The tendency of ascospores to break down and lose their identity in the ascus, when it was forcibly discharged from the perithecium, made it difficult to obtain an ascus with eight ascospores. The ascospores are ovoid in shape and measure 16 to 13μ by 15 to 19μ (Fig. 6).

It was observed that the initiation of perithecial formation occurs gradually and not only on specific species and varieties but also on specific plants. Fragaria chiloensis and F. ovalis plants were growing under the same conditions of temperature, light and humidity and were almost equally infected with powdery mildew, but the perithecia rarely occurred on leaves of F. ovalis and frequently occurred on leaves of F. chiloensis. Similarly, varieties 59-S-5-7 and Cambridge Vigour carried perithecia but no perfect stage was seen on the leaves of 59-S-5-6, Huxley and Talisman plants.

Another species of powdery mildew, Sphaerotheca pannosa, responds to environmental conditions in an identical way. Coyier and Hildebrandt (1) reported that some varieties of rose support the development of the perfect stage and others do not. They also reported overlapping descriptions of S. pannosa and S. humuli (S. macularis) in different monographs and this poses a difficulty in distinguishing these two fungi. However, S. pannosa frequently produces many more sexual bodies than S. macularis. Similar to S. pannosa, Erysiphe cichoracearum produces enormous numbers of perithecia on phlox.

It has also been observed that two plants of F. chiloensis growing side by side under exactly similar environmental conditions may have the sexual stage on one and only the asexual stage on the other. From these observations, the authors believe that, in the formation of perithecia by this fungus, physiological conditions of the host play a major role. This is substantiated by the fact that frequently strawberry plants either have perithecia on all three leaflets of a compound leaf or none of the leaflets have sexual bodies. The authors conclude that the changes brought about during the fall season in the months of October and November cause some physiological change(s) in the host which indirectly permits the production of perithecia by the fungus. However, this does not rule out the possibility that environmental factors directly influence the production of the perfect stage by the parasite.

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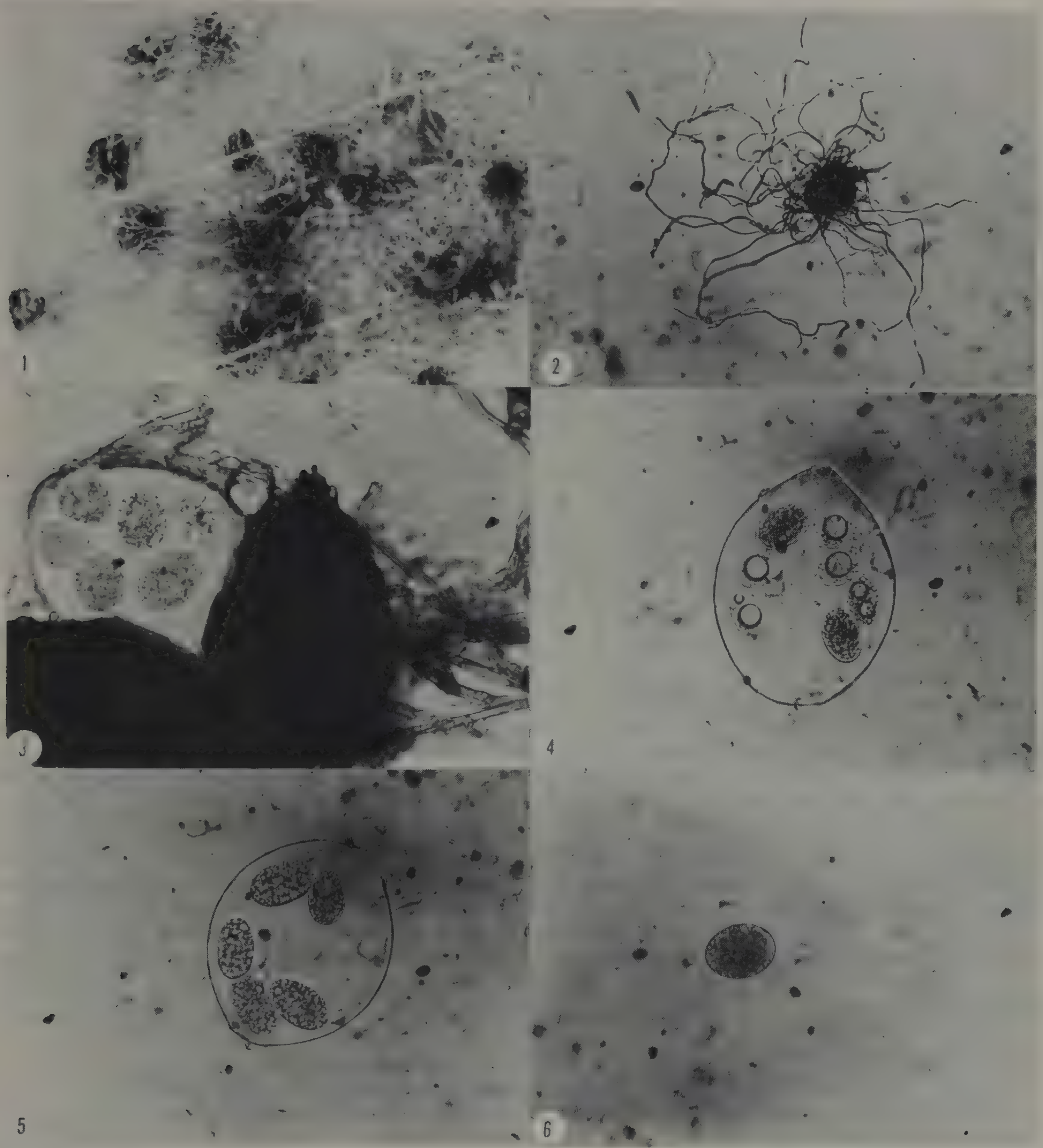

FIGURE 1. Perithecia, covered with a meshwork of white *S. macularis* mycelium, on the surface of a *F. chiloensis* leaf. FIGURE 2. A spherical perithecium with its long unbranched single appendages. FIGURE 3. A perithecium with its ascus emerging through its characteristic pie-shaped opening. FIGURE 4. An elliptical ascus with a thin apical zone and ascospores. FIGURE 5. An ascus showing a split near the apical zone through which the ascospores are released. FIGURE 6. A single ovoid ascospore.

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KERNEL-SPOT OF FILBERTS FOUND IN OREGON[1]

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A kernel-spot disorder of filberts very similar to one reported by Peglion in 1901 (2) from Italy was found in 1961 in a Nottingham filbert planting near Corvallis, Oregon. This disorder has apparently not been previously reported on filberts from the United States.

Filbert kernel-spot seems to be confined to the kernels; at any rate, infection of the shells has not been observed. The early stages of the disease are characterized by a "soft" type of lesion in the kernels. The mature lesions are distinct, circular to irregular, sunken and necrotic (Fig. 1). The pellicle of infected kernels generally disintegrates and sluffs off, and the surface of the nut becomes oily and feels slimy to touch.

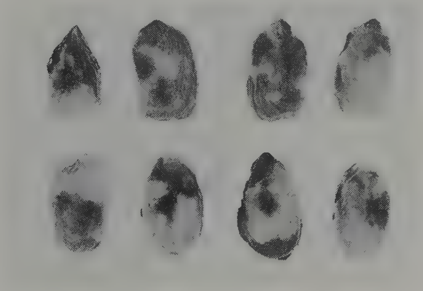

FIGURE 1. Kernel-spot of filberts. (Photographed by H. H. Millsap)

A microscopical examination of tissues taken from a number of the lesions revealed the presence of sexual and asexual spores of the yeast *Nematospora coryli* Pegl.; the same organism was subsequently isolated in a pure form from typical kernel lesions. However, while this organism was found in association with this disorder, it was not proved to be a primary parasite by infection experiments.

It is noteworthy that seed pitting of beans, known as "yeast spot," is also commonly accompanied by the presence of this organism (3). The yeast organism apparently enters through punctures made by lygus bugs (*Lygus* spp.) or green stink bugs (*Nezara* spp.). Stink and other similar bugs are also responsible for the kernel-spot disorder of pecans (1).

Possibly there is a similar relationship between *N. coryli* and stink bugs or lygus bugs and the development of kernel-spot of filberts, but definite proof of such a relationship is lacking.

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REDUCTION OF PEACH DECAYS BY HYDROCOOLING WITH CHEMICAL SOLUTIONS AND CHEMICALLY TREATED ICE

W. L. Smith, Jr. and W. H. Redit[1]

Summary

Sodium ortho-phenylphenate, sodium dehydroacetic acid, and mycostatin effectively reduced postharvest decays of peaches. However, the range of chemical concentration that effectively reduced decay without injuring the fruit was very narrow, particularly with sodium ortho-phenylphenate. When chemicals are used in ice refrigerated water to reduce peach decays, dilution by melting ice rapidly changes the chemical concentration. Therefore, practical methods of adjusting and maintaining these chemical concentrations are essential. Data show that ice containing a proper concentration of sodium ortho-phenylphenate adjusted to pH 11.5 maintained the concentration of that chemical quite uniformly without change in pH of the solution during 90 minutes of continuous hydrocooling. Incorporation of a chemical into the ice used for hydrocooling may be a simple and accurate method of maintaining uniform chemical concentrations during commercial hydrocooling.

INTRODUCTION

Many peach growers and shippers in the eastern and southeastern sections of the United States use ice-refrigerated flood-type hydrocoolers to remove field heat from the fruit before shipping in refrigerated cars or trucks. Studies have shown that peaches hydrocooled without chemicals in the water develop more decay during ripening than non-hydrocooled fruit held under the same conditions (2). Usually either sodium or calcium hypochlorite (100-120 ppm chlorine) is used in commercial hydrocooling operations to attempt to reduce the concentration of viable organisms in hydrocooling water and hence reduce postharvest decay. These chemicals have been shown to be only partially or irregularly effective in reducing decay (3, 4, 6, 9).

During the operation of ice-refrigerated hydrocoolers, melting ice dilutes any chemical added to the water. Most chemicals have an optimum concentration for decay reduction, above which they may be phytotoxic and below which they are only partially effective. If a chemical is to be adopted for the hydrocooling operation in the peach industry a relatively simple, rapid, and accurate method of determining its concentration is necessary and practical methods of adjusting the concentration of the toxicant in the solution must be worked out.

Accordingly, experiments were conducted to compare the relative effectiveness of three chemicals for reduction of peach decays and to test methods of determining and adjusting the concentration of one of these chemicals in hydrocooling solutions.

GENERAL MATERIALS AND METHODS

Peaches picked about 24 to 48 hours before use in the tests were obtained from the wholesale market in Washington, D. C. or from nearby orchards. Before each test, fruit was sorted for uniform size and maturity, and for freedom from decay, bruises, or other blemishes. The fruit usually required 2 to 3 days at 70° F to become fully ripe.

Fruit was artificially inoculated by dipping in a suspension of spores in beef peptone broth containing 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate). Uninjured fruit was inoculated with the brown rot organism (Monilinia fructicola (Wint.) Honey), while a shallow cut resembling a finger nail injury was made on each cheek of peaches inoculated with Rhizopus stolonifer (Fr.) Lind. The concentration of spores in the suspensions was 20 to 30 Monilinia spores or 70 to 80 Rhizopus spores per low power field of a microscope. For each test, 20 fruit were artificially inoculated with the respective organisms for each treatment. In addition, similar lots of fruit were not artificially inoculated.

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1958 TESTS

Peaches were dipped for 20 minutes in 32° F water containing chemicals previously shown to effectively reduce postharvest decay of peaches (2, 5, 7, 8). The ice for cooling the water was in polyethylene bags to prevent dilution of the chemical. Fruit temperatures after treatment averaged 53°. Fruit was then held at 50° in 4-quart tills for 3 days to simulate a transit period, then transferred to 75° to ripen. Decay readings were made when the peaches were removed from 50° and after 3 days at 75° when fruit was soft ripe.

No decay developed on fruit while it was held at 50° F. All the chemicals effectively reduced both Monilinia and Rhizopus decay at 75° (Table 1). A concentration of 0.1% sodium dehydroacetic acid was decidedly less effective than 1.0%.

Table 1. Postharvest decay of artificially inoculated peaches at 75° F with and without chemical additives in hydrocooling solutions.

Chemical additive and concentration %		No. of tests	% Monilinia decay after 3 days at 75° F: without chemical	with chemical	decay reduction	% Rhizopus decay after 3 days at 75° F: without chemical	with chemical	decay reduction
Sodium ortho-phenylphenate[a]	0.1	12	67	15	78	78	39	50
Mycostatin	0.01	12	67	11	84	78	43	45
Sodium dehydroacetic acid	1.0	5	83	13	84	97	43	56
Sodium dehydroacetic acid	0.1	5	56	39	31	78	45	42

[a] Sodium ortho-phenylphenate buffered to pH 11.5 with trisodium phosphate.

Of these chemicals sodium ortho-phenylphenate has been most thoroughly tested for reduction of postharvest peach decay and a residue tolerance of 20 ppm has been established by the Food and Drug Administration. Since dilution by melting ice during commercial hydrocooling affects the concentration of the chemical, the relation of chemical concentration to decay reduction was studied.

Both Monilinia and Rhizopus decay were most effectively reduced by a concentration of 0.5% sodium ortho-phenylphenate (Table 2), but much of the fruit was injured. Injured areas were brownish, mottled, and sometimes slightly sunken. The discoloration did not extend into the flesh of the fruit. Similar but less severe injury occurred on fruit treated with a 0.25% solution. No injury occurred at lower concentrations. Concentrations of 0.15 and 0.1% effectively reduced Monilinia decay but were relatively ineffective against Rhizopus decay. The chemical was relatively ineffective in reducing both types of decay at concentrations below 0.1%.

Table 2. Postharvest decay of artificially inoculated peaches at 75° F hydrocooled[a] with various concentrations of sodium ortho-phenylphenate[b].

% conc.	No. of tests	% Monilinia decay after 3 days at 75° F: without chemical	with chemical	decay reduction	% Rhizpous decay after 3 days at 75° F: without chemical	with chemical	decay reduction
0.5	9	85	4	95	97	39	62
0.25	5	80	14	80	98	53	46
0.15	5	80	31	60	98	78	20
0.10	9	85	39	54	97	74	24
0.05	9	85	55	35	97	68	30
0.025	5	80	69	13	98	84	14

[a] Peaches held 3 days at 50° F after hydrocooling, then transferred to 75°.
[b] Sodium ortho-phenylphenate buffered to pH 11.5 with trisodium phosphate.

Table 3. Concentration of sodium ortho-phenylphenate, pH change, and decay of peaches after 3 days at 75° F[a] as affected by melting commercial ice during successive runs in the same chemical hydrocooling solution.

		High natural inoculation[b]			Very high natural inoculation[c]		
		% decay in naturally	% decay in artificially inoculated fruit[d]		% decay in naturally	% decay in artificially inoculated fruit[d]	
% chemical concentration	pH	inoculated fruit	Monilinia inoculation	Rhizopus inoculation	inoculated fruit	Monilinia inoculation	Rhizopus inoculation
0 (Ice water control)	--	52	95	98	70	100	100
0.091[e]	11.6	18	80	63	45	100	95
0.082	11.5	13	76	59	45	100	93
0.076	11.4	13	65	68	60	98	98
0.073	11.0	14	79	54	55	98	100
0.065	10.9	10	76	56	85	100	98
0.061	10.7	11	73	52	52	98	98

[a] Fruit previously held 5 days at 50° F. [b] Average of four tests, 20 peaches per test.
[c] Average of two tests, 20 peaches per test. [d] Equals total decay - natural + artificial inoculation.
[e] Different lots of peaches hydrocooled for about 15 minutes in each run.

1960 TESTS

In 1960 a small ice-refrigerated flood-type hydrocooler was constructed for laboratory use. Water flow per unit of area and cooling rates of peaches were similar to those of commercial flood-type hydrocoolers. This hydrocooler was used to determine the effect of melting ice on the concentration of sodium ortho-phenylphenate, and methods were devised for testing and for adjusting the chemical concentration of hydrocooling solutions.

Several quick methods, devised by commercial concerns, were tested for the determination of concentrations of sodium ortho-phenylphenate in hydrocooling water. Under the conditions of our experiments, these were not sensitive enough to determine accurately changes in concentration caused by melting ice.

A modification of the titration method for the determination of phenols as originally described by Koppeschaar (1) was therefore used to determine the concentration of sodium ortho-phenylphenate in the hydrocooling solutions. The procedure was:

1. Place 50 ml of the hydrocooling solution in a 250-ml flask.
2. Pipette into the flask 75 ml of methyl alcohol, 10 ml of a 25% solution of potassium bromide, and 25 ml of a potassium bromide-potassium bromate solution (1.20% potassium bromide and 0.2784% potassium bromate).
3. Add 3 ml of concentrated hydrochloric acid, swirl, and let stand for 10 minutes.
4. Add 3 ml of a 20% potassium iodide solution; an intense red color is produced by the free iodine.
5. Titrate immediately with a standardized solution of sodium thiosulfate, approximately 0.1 N until solution becomes straw colored. Add a few drops of soluble starch indicator solution and titrate to a colorless endpoint.

The concentration of sodium ortho-phenylphenate in percent is calculated by subtracting the titer of the sample from the titer of the blank (50 ml of distilled water instead of 50 ml of hydrocooling solution) and multiplying the difference by 0.132 x normality of the sodium thiosulfate solution.

The method is cumbersome and for accurate analyses competent, trained personnel must be available. Preparation of the test solutions is time consuming and exacting. About 15 minutes were needed to determine the concentration of sodium ortho-phenylphenate. Such a process would be impossible for commercial use since the concentration of the chemical in solution in the hydrocooler tank would have changed before the sample could be analyzed.

At the start of a test, water was cooled to about 32° F. Then enough sodium ortho-phenylphenate and sodium hydroxide were added to form a solution containing approximately 0.1% sodium ortho-phenylphenate with pH 11.5.

Baskets of naturally inoculated peaches and those artificially inoculated with Monilinia or Rhizopus spores then were cooled simultaneously until the fruit temperature averaged about 45° F. The baskets of fruit were placed at 50° for 5 days and then transferred to 75°. Decay

Table 4. Concentration of sodium ortho-phenylphenate, pH change, and decay of peaches after 3 days at 75° F[a] as affected by melting of ice containing sodium ortho-phenylphenate (chemical ice) during successive runs in the same chemical hydrocooling solution.

		Low natural inoculation b			Very high natural inoculation[c]		
		% decay in naturally inoculated fruit	% decay in artificially inoculated fruit[d]		% decay in naturally inoculated fruit	% decay in artificially inoculated fruit[d]	
% chemical concentration	pH		Monilinia inoculation	Rhizopus inoculation		Monilinia inoculation	Rhizopus inoculation
0 (Ice water control)	--	7	88	100	66	94	99
0.088[e]	11.6	2	25	75	58	89	69
0.093	11.7	2	35	53	48	88	68
0.092	11.6	7	33	82	48	89	61
0.092	11.4	3	30	68	44	85	53
0.087	11.3	20	33	58	39	88	65
0.086	11.2	0	30	57	39	95	74

[a] Fruit previously held 5 days at 50° F. [b] Average of three tests, 20 peaches per test.
[c] Average of four tests, 20 peaches per test. [d] Equals total decay - natural + artificial inoculation.
[e] Different lots of peaches hydrocooled for about 15 minutes in each run.

readings were made on removal from 50° and after 3 days at 75°.

The same solution was used in six runs, each run lasting about 15 minutes. Ice was added after each run, or at times during the run when the solution temperature went above 34° F. The concentration of sodium ortho-phenylphenate was determined at the end of each run.

Two types of ice to cool the water were compared. In one series of tests commercial egg-sized water ice was used. In the other an ice was made by freezing solutions containing 0.1% sodium ortho-phenylphenate adjusted to pH 11.5 with sodium hydroxide (chemical ice). Sufficient bromthymol blue was added to the solutions before freezing to give the ice a blue color as a safety precaution. This ice was broken to about egg size before adding to the hydrocooling solution. When chemical ice was to be used, the remaining commercial ice previously used to cool the solution was removed.

Hydrocooling Tests with Commercial Ice: Melting ice continuously reduced the concentration of sodium ortho-phenylphenate and lowered the pH of the solutions (Table 3). After 90 minutes of hydrocooling, the concentration of sodium ortho-phenylphenate was reduced from 0.091% to 0.061% and the pH lowered to 10.7.

When 52% of the naturally inoculated fruit hydrocooled in ice water without a chemical additive (control) developed decay, the addition of sodium ortho-phenylphenate very effectively lessened the percentage of decay. When natural infection was considerably higher (70%), decay was only slightly reduced by the chemical treatment.

Total decay was excessively high in plain ice water cooled fruit that had been artificially inoculated with either Monilinia or Rhizopus spores (Table 3). Hydrocooling with the sodium ortho-phenylphenate solution reduced decay of artificially inoculated fruit below that of the controls only when the percentage of natural infection that developed on the fruit treated with sodium ortho-phenylphenate was relatively low. Even in these cases the total percentage decay was high. When the percentage natural infection in the treated fruit was high the chemical treatment had no effect on total decay in the artificially inoculated fruit. In either case the concentration of the chemical had little effect on the amount of decay that developed.

Hydrocooling Tests with Chemical Ice: When ice containing sodium ortho-phenylphenate was used to hydrocool fruit, the concentration of that chemical in the hydrocooling solution remained relatively constant and the pH of the solution was only slightly lowered (Table 4). In three tests the decay due to natural inoculation was low (7%) in the controls and usually considerably reduced when fruit was hydrocooled with sodium ortho-phenylphenate. In the other four tests natural infection was very high in the controls (66%) and only slightly to moderately reduced by the chemical treatment.

Total decay in the ice water control was excessively high when the fruit was artificially inoculated (Table 4). Where natural infection was low, total decay in Monilinia inoculated fruit was effectively lessened by hydrocooling in sodium ortho-phenylphenate and in Rhizopus inoculated fruit usually was moderately reduced. When natural infection was high, the fungicidal treatment had little effect on Monilinia inoculated fruit. Decay was reduced slightly in the Rhizopus inoculated fruit.

DISCUSSION

Rapid cooling of peaches to about 50° F and maintaining such a temperature during transit is sometimes considered to provide decay control even after the fruit is removed from the refrigerated vehicle to higher temperatures. Data presented show that peaches hydrocooled to about 45° and held 5 days at 50° often developed 100% decay within 3 days after the peaches were transferred to 75°. Growth of the organisms, therefore, was inhibited only during the period at low temperature. Effective control of decay at higher temperatures requires a treatment that will destroy or substantially reduce the number of decay-producing organisms. Some of the possibilities and the shortcomings of postharvest fungicides for the reduction of decay during marketing are shown.

In 1958 sodium ortho-phenylphenate, mycostatin, and sodium dehydroacetic acid used as postharvest fungicides in hydrocooling water were almost equally effective in reducing decay due to Monilinia fructicola or Rhizopus stolonifer. The concentration of the chemicals in solutions, particularly of sodium ortho-phenylphenate, was extremely important in obtaining effective decay reductions with no phytotoxicity. Such tests indicate the importance of measuring concentrations of chemicals in hydrocooling solutions and the need for a practical method of analyzing the solution that is accurate and simple enough to be used by average commercial operators.

In the 1960 tests the concentration of sodium ortho-phenylphenate was reduced approximately one third by melting ice during 90 minutes of hydrocooling. In commercial operations a hydrocooling solution may be used continuously for several hours and possibly not changed for a week. Under such conditions it is conceivable that dilution of the chemical by melting ice could lower its concentration to a point where almost no decay reduction would be obtained. The decrease in pH over the limited period of these tests also indicates that pH of commercial hydrocooling solutions could be lowered to the point where sodium ortho-phenylphenate is phytotoxic.

Ice containing the desired concentration of this chemical and buffered to the proper pH was very effective in maintaining a relatively uniform concentration of the chemical in the hydrocooling solution over a period of repeated use. Because public safety would require identification of this chemical ice when used commercially, a color approved by the Food and Drug Administration could be added.

The data show some of the limitations of a postharvest fungicide. Too often it is assumed that a postharvest fungicide will effectively reduce decay during marketing regardless of the amount of incipient decay on the fruit before packaging and shipment. Data presented here indicate that when incipient decay was extremely high, sodium ortho-phenylphenate was ineffective in reducing decay. On the other hand, when it was moderate or low, postharvest decay during a simulated transit and marketing period was effectively reduced. Too often peaches with low percentages of incipient decay may be exposed to heavy concentrations of pathogenic fungus spores during picking and packaging operations. The buildup of fungus inoculum in hydrocooling water without an effective toxicant is a distinct possibility. Inoculation shortly before treatment with fungicides, as reported in these tests, simulates such postharvest packinghouse inoculation. The benefits of a postharvest fungicide under such circumstances is demonstrated. Postharvest treatments to reduce decay, therefore, should be considered as supplements to, and not as substitutes for, proper decay-control practices in the orchard.

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AN EVALUATION OF TWO FUNGICIDES AS SOIL TREATMENTS WITH AND WITHOUT HERBICIDE FOR TOMATO FRUIT DISEASE CONTROL[1]

D. F. Crossan and A. L. Morehart[2]

Summary

Two fungicides were evaluated for use as sprays on the soil in an effort to reduce tomato anthracnose (Colletotrichum phomoides) and fruit rot (Rhizoctonia solani). The fungicides used were 80% maneb and 50% 5-chloro-4-phenyl-1,2-dithiol-3-one (Hercules 3944).

Maneb sprayed on the soil surface around tomato plants at the rate of 4 pounds per acre significantly reduced anthracnose and Rhizoctonia fruit rot. The addition of the herbicide 76% propyl ethyl-N-butyl thiol carbamate (Tillam) did not increase or decrease the effectiveness of maneb for disease control. Nearly perfect anthracnose control was obtained with eight applications of maneb applied to the foliage and fruit in a standard spray program.

Hercules 3944 had no effect on either anthracnose or Rhizoctonia at the rate used.

INTRODUCTION

Attempts to control tomato diseases such as anthracnose (Colletotrichum phomoides) or fruit rot (Rhizoctonia solani) by application of fungicides to the surface of the soil have met with varying success. Wilson, et al. (2) applied 76% ziram (Zerlate) to the soil and were able to reduce slightly the incidence of tomato anthracnose. Crossan, et al. (1) reported reduction of Rhizoctonia fruit rot of tomato when 80% maneb or Tribasic Copper Sulfate, alone or in combination, was applied to soil in greenhouse flats.

With the advent of potentially useful herbicides in tomato culture, the application of fungicides to the soil surface needs more study. In theory, the inoculum potential of pathogens such as C. phomoides, Alternaria solani, and R. solani that survive in the soil should be reduced when the soil surface is sprayed with an effective fungicide. Further, if soil disturbance thereafter was reduced by use of an herbicide, any such fungicidal effect might be enhanced. To test this idea, an experiment was conducted wherein two chemicals, 80% maneb (Dithane M-22) and 50% 5-chloro-4-phenyl-1,2-dithiol-3-one (Hercules 3944), were used as soil treatments alone and in conjunction with 76% propyl ethyl-N-butyl thiol carbamate (the herbicide Tillam), and contrasted with a specific number of foliar sprays of maneb.

MATERIALS AND METHODS

The plot area received 600 pounds of 0-20-20 fertilizer turned under with a rye cover crop. Plants were set on May 15 and side-dressed with ammonium nitrate on May 30, June 10, and June 25 to give a total of 90 pounds of nitrogen per acre.

A split-plot design was used wherein six fungicidal treatments were compared under herbicide or no-herbicide conditions. Each whole plot consisted of three rows of the tomato variety Del 14-2, 30 feet in length, and 48 inches apart. Each plot was replicated four times. Tillam was sprayed on the soil surface of half of each plot at the rate of 1 gallon of commercial product per acre. The plot was then cultivated to disperse the herbicide into the soil. Immediately thereafter, either 80% maneb or Hercules 3944 was applied to the soil of the whole plot area at a rate calculated to give 4 pounds of formulated material per acre. Specific plots were also designated to receive foliar applications of maneb. The final comparison of soil treatment, with and without herbicide and/or foliar sprays, and the dates and rate of applications were as follows:

1. Untreated.
2. Maneb; (1 1/2-100); eight foliar sprays on June 30, July 11, 18, 25, and August 1, 8, 15, and 25.

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[2] Respectively, Associate Professor and Research Associate, Department of Plant Pathology.

3. Maneb; 4 lb/acre applied to soil on June 29.
4. Maneb; 4 lb/acre applied to soil on June 29, followed by three foliar sprays (1 1/2-100) on July 11, 25 and August 8.
5. Hercules 3944; 4 lb/acre applied to soil on June 29.
6. Hercules 3944; 4 lb/acre applied to soil on June 29 followed by three foliar sprays of maneb (1 1/2-100) on July 11, 25 and August 8.

Non-herbicide plots were cultivated as needed until mid-August.

Fruit were picked from the center row of each 3-row plot on August 8, 18, and 31 and graded into marketable and disease categories.

RESULTS

The data obtained are presented in Table 1. A period of low rainfall in the latter part of July and the first part of August contributed to a generally low incidence of anthracnose (4 to 5% of the total fruit picked) and Rhizoctonia fruit rot (about 10%). However, several interesting results were obtained:

(a) The herbicide Tillam gave excellent weed control for about a 6-week period. The combined soil treatment of Tillam and maneb resulted in a reduction in weight of marketable fruit in each instance where the combination occurred as soil treatments.

(b) There was no significant effect on the incidence of anthracnose due to the use of Tillam.

(c) All maneb treatments significantly reduced anthracnose. It was of particular interest to note that maneb applied at 4 pounds per acre with no subsequent foliar applications of fungicide significantly reduced anthracnose development.

(d) Hercules 3944 did not control anthracnose or Rhizoctonia fruit rot at the rates used. The use of three sprays of maneb superimposed over this treatment resulted in significant anthracnose control.

Table 1. Comparison of soil and foliage treatments, with and without herbicide, for tomato disease control.

Treatment	Av. no marketable fruit (pounds)	Av. no. fruit graded cull	
		Anthracnose	Rhizoctonia
1. Check	73.0	8.25	22.7
Check and herbicide	72.5	13.00	24.2
2. Maneb (8 sprays)	77.0	0.05	19.0
Maneb and herbicide	72.0	0.05	19.7
3. Maneb (4 lb/A)	81.7	2.75	14.5
Maneb and herbicide	61.0	3.50	10.5
4. Maneb (4 lb/A) plus 3 sprays	87.7	1.00	20.5
Maneb and herbicide	74.2	0.05	14.0
5. H-3944 (4 lb/A)	78.2	12.00	22.7
H-3944 and herbicide	69.2	9.50	22.2
6. H-3944 (4 lb/A) plus 3 sprays maneb	68.7	1.50	21.5
H-3944 and herbicide	72.5	2.50	19.5
LSD .05	12.2	5.30	7.8

CONCLUSIONS

The use of maneb as a soil fungicide offers considerable promise in the control of tomato anthracnose and Rhizoctonia fruit rot. The use of this fungicide on the soil surface could reduce the total number of spray applications necessary for anthracnose control and would also reduce fruit rot due to Rhizoctonia. However, the effect of an application of maneb to the soil upon such foliar disease as early blight (Alternaria solani) and gray leaf spot (Stemphylium solani) must also be evaluated. The addition of Tillam did not affect the efficiency of anthracnose disease control by maneb when this fungicide was used solely on the soil, as a foliar spray, or a combination of both. The evidence indicates a suppression of yield when the two compounds were applied to the same soil site.

The hypothesis that maneb or other fungicides could be used to advantage in combination with an herbicide should be tested further on a variety of soil types and under more severe disease conditions than existed in the present experiment.

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DR. THOMAS TAYLOR AND THE DELINTING OF COTTON SEED

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Dr. Thomas Taylor, microscopist of the United States Department of Agriculture, 1871 - 1895, dabbled in all manner of biological problems during his curious career without achieving lasting fame in any. He was unique in the unanimity with which his numerous publications have been consistently ignored by later workers in all of the fields into which he ventured.

There is at least one phase of his work, however, for which he should be given proper credit, heretofore denied him. He was very fond of using sulphuric acid in his operations, particularly in connection with studies of diseased plant material, a habit which certainly contributed heavily to his failure to obtain worthwhile results as a pioneer phytopathologist. The Doctor and his favorite chemical should have scored heavily, however, with the publication in 1881 of his experiments on delinting cotton seed.

In a paper presented before the American Association for the Advancement of Science at its Boston meeting in 1880 and later published in the proceedings of the same organization (7), a clear-cut report was made of the results obtained from sulphuric acid treatment of seed cotton and other hard seeds. Particular attention was given to the effect on cotton seed and it was emphasized that the acid treatment removed the lint rapidly and effectively without affecting the germinating power of the seed. Treated seed germinated in approximately half the time needed for untreated seed and normal plants resulted. The process also made it possible to skim off "light, inferior, and wormy seeds." Treated seed could be planted by machine, less seed was necessary for planting, and there were other obvious advantages.

The germination tests were made in a Department greenhouse under the general direction of Prof. Wm. Saunders, Horticulturist and Superintendent of Gardens and Grounds, which gave the experiments an aura of good scientific standing which might not have been the case otherwise.

It may be noted that Dr. Taylor overlooked entirely the possibilities of disease control presented by his new method of seed treatment. The fact of seed-borne diseases in general was outside the range of his thinking and his knowledge of cotton diseases in particular was negligible.

William G. Le Duc, the Commissioner of Agriculture,. in his report for 1880 (5) discusses the new process briefly and includes a description of the recommended method supplied by Dr. Taylor. The latter also issued privately (8) at this time what was in effect a press notice covering his work including a reprint of a letter from Prof. J. L. Budd of the Iowa Agricultural College which was originally published in a New York paper. Prof. Budd stated that "Thomas Taylor's discovery... had been pronounced by leading European journals the most useful work performed by our Department of Agriculture." Wm. Saunders' report on the germination tests was included.

As far as the present writer has been able to find, the Taylor method of delinting cotton seed was forgotten from this point on. About 1911 Prof. J. F. Duggar and his associate E. F. Cauthen (4) of the Alabama Agricultural Experiment Station in the course of experimental work on boll rot of cotton used sulphuric acid as one of several treatments tried. No details of the process used were given and Dr. Taylor's work was not mentioned. Since that time there have been many reports of work with the sulphuric acid process in connection with disease prevention studies and various phases of cotton culture. All publications on the subject which it has been possible to review, and they are many, are unanimous in ignoring Dr. Taylor's pioneer efforts with sulphuric acid and either credit the process to Duggar and Cauthen, citing Bulletin 153 (4), or ignore the past.

Brown and Gibson (3) in particular name Duggar as the originator of the process without, however, giving a definite literature reference. They quote Duggar as saying "the following method of delinting cotton seed with sulphuric acid has been used in the Botany Division here during the past three years." The simple method recommended by Duggar is then quoted in a brief paragraph, the authors themselves adding "until the present time, so far as the writers are aware, the method described by Duggar for delinting cotton is the only one that has been published."

The source of the quotation from Duggar has not been found. It is not in Bulletin 153, which is usually cited by other workers as the original publication on the subject. Bain (1), Quintanilha, Cabral, and Quintanilha (6), and Barker, et al. (2) review the subject in considerable detail with an extensive bibliography in each case. These authors and those cited in the three publications often refer to Duggar or Duggar and Cauthen, but never to Taylor. It would appear to be a case where retrieval of scientific information, a subject prominent in the scientific world today, has been faulty.

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CONTROL OF PECAN SCAB IN ALABAMA IN 1961 AND ITS RELATION TO NUT QUALITY[1]

Urban L. Diener

Summary

Dodine, zineb, and ziram were evaluated as protectants on Schley and Success pecans for control of pecan scab in Alabama in 1961, a severe scab year. Dodine gave excellent disease control, zineb was rated good, and ziram poor when evaluated by scab index from green nuts. Data on quality of nuts at harvest were closely correlated with scab indices on Success pecans in Baldwin County.

INTRODUCTION

Alabama pecan growers have averaged 4.4 million dollars annual income during the past 10 years, with a high of 10 million dollars in 1958. Pecan scab, caused by Fusicladium effusum Wint., is a limiting factor in pecan production even with improved varieties. Despite the data and recommendations of Cole and other investigators (1, 2, 3, 6, 13, 17) that scab could be controlled by fungicidal applications, spraying had not become widespread in Alabama and other States before 1960. However, with Cole's (7) report in 1960 of scab control using dodine, an increased interest in disease control has been evident among pecan growers. Another factor has been the improved models of air-blast sprayers now available, which give adequate coverage, with limited manpower, of large pecan trees.

The investigations reported here not only evaluate several fungicides for pecan scab control in Alabama but also present data on nut quality at harvest. The uniformity of Success pecan trees in the Baldwin County experiment appeared adequate for such a study.

MATERIALS AND METHODS

Fungicidal spray experiments begun in 1960 were discontinued after three applications because of poor nutset. Experiments on pecan scab control were established on a more extensive scale in 1961 with cooperators in Autauga, Baldwin, and Mobile counties, using their high-pressure (hydraulic) or air-blast sprayers to apply dodine (Cyprex), zineb (Dithane Z-78, Parzate C), and ziram (Zerlate) on Schley and Success varieties. Seven or eight applications were made at approximately 3-week intervals from prepollination (about April 25) to about August 25. Phosphate insecticides (parathion, malathion, or methyl parathion) were combined with part or all of the applications varying with the cooperators' insect problems. DDT was also added to the last spray or two at most locations. Check plots were sprayed with insecticide only. High pressure spray machines (John Bean) were operated at about 450 to 600 psi. The air-blast sprayers were John Bean Speed Sprayers Model 502CP. The sprayers circled Schley trees (40 to 50 years old) in Autauga County, whereas Success trees (15 years old) in Baldwin County were sprayed from two sides. Single tree plots were replicated three, eight, or ten times for each treatment varying with location.

The high-pressure spraying experiment in Mobile County was a rate x frequency-of-application study with dodine. Success trees (35 years old) were sprayed with 1/2, 1, and 1 1/2 pounds of dodine per 100 gallons of spray for six, four, and two applications, respectively. Data were taken from three replications.

Data for scab index ratings were taken just before shuck-split (Sept. 21-26) from 50 green nuts randomly collected from each tree. Nuts were graded into five classes on the basis of percentage of scabbed surface of the green shuck. These classes closely parallel the standard previously reported (15, 16) for grading the degree of scab infection on the husks. Thus: 0, no scab; 1, trace to 10%; 2, 11 to 25%; 3, 26 to 50%; 4, 51 to 100%. The index rating was calculated by multiplying the number of nuts in a class by the class number. The products for all classes were totaled and divided by 50, which gave the scab index for a plot. Mean index of each treatment was then calculated.

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Data on nut quality of Success pecans were collected by the following procedure. Trees were shaken and a random sample of 100 nuts collected by picking up 12 to 13 nuts at eight different places under the tree. Nuts were weighed and the number per pound calculated. Each of the 50 lots of 100 nuts was cracked, and data were taken for the number of pops (culls), total weights of all meats as percentage of in-shell weight, weight of No. 1 meats as percentage, weight of ambers as percentage, and the price offered for the combined meats by a commercial sheller. Data were summarized as means for ten replications for each treatment.

RESULTS AND DISCUSSION

Environmental conditions were highly favorable for disease development during the 1961 growing season; it was a very severe scab year. For the first time many growers observed the Stuart variety to scab severely in Baldwin County. The yield of unsprayed trees of the Schley, Mahan, and other highly susceptible varieties was reduced 75 to 90% by scab at many locations. Only a light incidence of scab was observed in the Mobile experimental area. However, powdery mildew was quite severe at this location.

Disease Control Data: The results are summarized in Table 1. A scab index of less than 1.0 indicates that approximately 90% of green nuts had less than 10% of the shuck surface covered with scab infections, which constitutes a high degree of control. Dodine at 1/2 or 1 pound gave excellent control of scab in 1961. Zineb also gave good control when applied by air-blast sprayers. Ziram gave relatively poor disease control at all locations.

Table 1. Fungicidal control of scab on Schley and Success pecans at several locations in Alabama in 1961 using air-blast and high-pressure sprayers.

Treatment	Rate/100 gal. (in pounds)	Data as disease indices			
		Autauga (Schley)		Baldwin (Success)	Mobile
		air-blast	high-pressure	air-blast	high-pressure
Dodine	1	0.71[a]	-	0.70	0.08[b]
Dodine	1/2	-	2.10	0.67	0.41[b]
Zineb	2	1.76	2.47	1.46	-
Ziram	2	2.43	3.84	2.78	-
Check (insecticide only)	--	3.98	4.00	3.57	1.49
LSD 1%		0.86	1.90	0.49	0.85

[a] Scab index: 0, no scab; 1, trace to 10%; 2, 11 to 25%; 3, 26 to 50%; 4, 51 to 100%. Data from 50 green nuts per tree replicated ten times.

[b] 1-pound rate - four applications, 1/2 rate - six applications, all other treatment results were from eight applications.

The relatively poor control with the high-pressure sprayer in Autauga County could have been the result of equipment differences, but it is probable that the 1/2-pound level of dodine was too low under high disease incidence. Also, the seven applications were made at rather long intervals in May, June, and July, being 30 rather than 21 days apart.

In the rate x frequency-of-application of dodine experiment at Mobile, disease control was good from both 1/2 pound x 6 sprays, and 1 pound x 4 sprays (Table 1). The 1 1/2 pounds of dodine for 2 sprays (not shown in Table 1) gave a scab index of 0.65, which was rather poor control since disease incidence was light in these plots. Observations indicated considerable premature nut drop from scab with the two spray treatment.

The highly effective control of pecan scab with dodine in Alabama is similar to that reported by Cole (7, 8) in Georgia and Barnes (1) in Oklahoma. Zineb is recommended for pecan scab control in most southeastern States, and by United States Department of Agriculture investigators (15). These data show that zineb gave good scab control, but was not so effective as dodine under Alabama conditions in 1961. Other recent data (8, 9, 10) indicate a similar pattern. However, zineb was superior to dodine for control of several other foliage diseases; the importance of these diseases as well as the variety of pecan in a given area may predicate the choice of the fungicide. Although Cole (4, 5) initially reported good results with ziram, these data as well as other (1, 7) indicate it is frequently ineffective for control of pecan scab.

Table 2. Relation of pecan scab control with fungicides to nut quality and price of Success variety in Baldwin County, Alabama in 1961.

Treatment	Green nuts			Grade at harvest						Yield
Fungicide (pounds/100 gal.)	Nuts/ pound	Disease index	Shucks scabbed 0-10%	Nuts/ pound	No. pops	Total wt. meats (%)	Wt. No. 1 meats (%)	Wt. of ambers (%)	Offered price (cents)	Est. loss premature drop
Dodine, 1	17.2	0.70	89.4%	50.7	4.3	49.3	39.0	10.3	19.4	---
Dodine, 1/2 and 1[a]	16.4	0.67	88.0	50.0	5.0	44.7	34.5	10.8	17.0	---
Zineb, 2	16.8	1.46	58.8	54.2	6.1	47.5	35.5	11.9	17.9	2%
Ziram, 2	18.0	2.78	6.2	53.6	4.5	46.4	35.5	10.8	17.9	10%
Check[b]	21.2	3.57	4.8	56.7	7.2	44.5	29.2	15.2	15.1	30%
LSD 1%	1.5	0.5		4.5	4.4	3.7	6.3	5.4	2.6	

[a] First four sprays 1/2 pound; last four sprays 1 pound.

[b] Insecticide only.

Ziram and summer oil emulsion used by itself or following Bordeaux mixture in the early sprays has given good control of scab in Florida (13, 14).

Nut Quality Data: Data on nut quality taken at harvest on Success pecans in Baldwin County are summarized in Table 2. The number of green nuts per pound, scab index, and percentage of shucks with less than 10% scabbed surface are shown in the green nut data. The data on quality or grade are well correlated with disease data, although the best treatment is not always significantly better statistically than the check (unsprayed trees).

The 1-pound dodine treatment was significantly better than the check at the 1% level in the number of nuts per pound, total weight of meats, weight of No. 1 meats, and price offered by a commercial sheller. The lower level of dodine (1/2 pound - 4 sprays, 1 pound - 4 sprays) gave significant increases only in the number of nuts per pound. These are correlated with the disease data from green nuts and together show that the superior scab control of dodine also resulted in higher nut quality.

Zineb gave significant increases for weight of No. 1 meats and in offered price when compared with the check. Zineb also gave good disease control. Ziram data were significant in weight of No. 1 meats and offered price when compared with the data from unsprayed trees. In nut quality ziram data were similar to zineb, although disease data show ziram to be considerably less effective in scab control on the shucks.

Data from scab control experiments through the years have generally consisted of the degree of scab infection, a quality measurement such as the number of nuts per pound, and either yield or percentage of marketable nuts per treatment. Extensive data on the grade of nuts from the fungicidal treatments have been reported in only a few instances. Boyd (2) in 1927 made comparisons of grade and market value of nuts harvested from fungicidal control experiments of pecan scab in Georgia on trees 9 to 18 years of age. Large, in 1952-53 (11, 12), took data on percentage of kernels and culls from 100-nut cracking samples as well as number of nuts per pound, yield, and percentage of marketable nuts. Quality data in Table 2 are the basis for the price offered by a commercial sheller for a given lot of pecans averaged for 10 random samples for each treatment. These data show that disease control prevented losses in quality of pecan and in price as well as losses in yield from premature drop (see Table 2). With a more susceptible variety, such as Mahan or Schley, the return from disease control in both yield and quality would be far greater, since unsprayed orchards of these two varieties, observed in 1961, averaged 75 to 90% losses. The grower-cooperator orchards with the more susceptible varieties in 1961 experiments lacked the uniformity judged necessary for a quality or yield study. This investigation has demonstrated the value of the control of pecan scab with fungicidal sprays in increased returns in quality as well as total production.

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AN EVALUATION OF CERTAIN FUNGICIDE - ADJUVANT COMBINATIONS FOR CONTROL OF PECAN SCAB

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Abstract

During the 1960 season, four fungicides, singly and in combination with an adjuvant, were sprayed onto pecan trees at 2- and 3-week intervals for control of pecan scab. Each of the fungicides alone and the fungicide-adjuvant combinations provided commercial control. The addition of an adjuvant did not improve the efficiency of any of the fungicides. Zineb and dodine fungicides are recommended for control of pecan scab in Oklahoma.

INTRODUCTION

Pecan scab, caused by the fungus *Fusicladium effusum* Wint., limits the production of nuts in the major pecan producing States. Control of this serious disease is of great importance to the pecan industry. When pecan trees are not sprayed efficiently with effective fungicides during seasons when rains and long dew periods occur frequently, nuts of most of the improved varieties, and nuts of some seedling or native trees, are lost because of scab.

Bordeaux mixture, ziram, zineb, and dodine are recommended for scab control in the southeastern States (5, 9, 10). The use of Bordeaux mixture in the southwestern States causes injury during hot, dry weather and also causes aphid and mite populations to increase (12). Ziram is frequently ineffective during wet periods in both areas (3). In Texas and Oklahoma zineb is the currently recommended fungicide for scab control (2, 3, 12). Recently two other types of organic fungicides, dodine (Cyprex[2]) and 2, 4-dichloro-6-(*o*-chloroanilino)*s*-triazine (Dyrene[2]), have been reported very effective for scab control (1, 2, 4, 5).

During seasons with frequent rains and many long dew periods it is advisable to spray susceptible trees every 2 weeks to insure adequate protection. As many as five to nine applications may be required to achieve commercial control. Many Texas and Oklahoma pecan growers consider such a program too costly. Fungicide formulations that weather and adhere to foliage better are needed to decrease the number of applications required. Particles of zineb carry a negative electrokinetic charge and don't adhere as well to negatively charged foliage as do the positively charged particles of Bordeaux mixture (11). In addition, deposits of zineb deteriorate rapidly 8 to 10 days after application (8). Spray adjuvants are needed that will either prolong or increase the activity of fungicides by providing better coverage, increasing deposition, or by synergistically increasing the activity of the fungicide itself. Such adjuvants would reduce the required number of applications of fungicides to a more economically attractive figure.

The primary objective of the presently reported test was to test several adjuvants for increasing the effectiveness of pecan fungicides. The second objective was to obtain additional data on the relative effectiveness of dodine (Cyprex) and Dyrene in comparison with recommended zineb fungicides. Another objective was to run a preliminary field trial of a zineb-nickel chloride mixture (Rohm and Haas 0-3818B). Such a mixture has shown some promise as both an eradicant and a protectant fungicide for control of leaf and stem rust of wheat (7).

MATERIALS AND METHODS

During the 1960 season four effective pecan fungicides were field tested individually and in combination with seven spray adjuvants (Table 2). When tested individually, fungicides were applied at 2- and 3-week application intervals beginning in May and ending in August. Fungicide and adjuvant combinations were applied every 3 weeks during the same period. Most of the treatments were made on 30-year-old Western pecan trees. Because of insufficient trees, some of the treatments were made on Squirrel trees of the same age in the same orchard. Both varieties are susceptible to scab. The fungicides and adjuvants used are listed in Tables 1 and 2, respectively. Rates at which each material was used are presented in Tables 3 and 4.

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[2] Mention of a trade name does not constitute a guaranty or warranty of the product named and does not signify that it is approved to the exclusion of other comparable products.

Table 1. Active ingredients and manufacturers of commercial fungicides used in pecan scab control trials in Oklahoma during 1960.

Fungicide	Active ingredients	Manufacturer
Parzate	65% zineb	E. I. du Pont de Nemours & Co., Inc.
Parzate C	65% zineb	E. I. du Pont de Nemours & Co., Inc.
Dithane Z-78	75% zineb	Rohm & Haas Co.
Cyprex 65-W	65% dodine	American Cyanamid Co.
Dyrene 50-W	50% 2,4-dichloro-6-(o-chloroanilino)-s-triazine	Chemagro Corp.
0-3818B	2.8 parts zineb + 1.0 part $NiCl_2 \cdot 4H_2O$	Rohm & Haas Co.

Table 2. Formulations and manufacturers of spray adjuvants used with fungicides for pecan scab control trials in Oklahoma during 1960.

Adjuvant	Formulation	Manufacturer
Du Pont Spreader -Sticker	88% sodium sulfates of mixed long chain alcohol-fatty acid esters and diethylene glycol abietate in water.	E. I. du Pont de Nemours & Co., Inc.
Plyac Spreader -Sticker	35% emulsifiable A-C Polyethylene, fatty acid amine condensate and an alkyl aryl sulfonate in an unspecified solvent.	General Chemical Division, Allied Chemical Corp.
Good-rite p.e.p.s.	50% Polyethylene polysulfide.	B. F. Goodrich Chem. Co.
National Sticker	40% benzyl sucrose, cyclic acetals of sucrose, propylidene and butylidene in methanol.	Holloway Sucro-Chemicals Corp.
Triton B-1956	77% modified phthalic glycerol alkyd resin in ethylene dichloride.	Rohm & Haas Co.
Crag Glyodin	30% 2-heptadecyl glyoxalidine acetate in isopropanol.	Union Carbide Chemicals Co.
Esso LO-3328	Emulsifiable clear mineral oil.	Esso Research and Engineering Co.

Spray applications were made with a conventional hydraulic spray machine operating at a pressure of 600 psi. The trees were individually circled and approximately 20 gallons of spray material were applied to each tree on each spraying date. All possible combinations were not tested because of lack of sufficient trees. All adjuvant combinations involving zineb were made, but not on any one variety or with a single zineb fungicide. The treatments used on each variety are presented in Tables 3 and 4. A special effort was made to insure complete coverage at every spraying, as poor coverage can result in poor control by even the most effective fungicide. Randomized, single-tree plots of 30-year-old Squirrel and Western pecan trees were replicated four and five times, respectively.

Rain and long dew periods occurred frequently during the entire season, creating favorable conditions for infection of nuts and foliage. Between April 1 and September 30, 1960, 28.7 inches of rain fell in the grove during 37 rains (0.01 inches and over).

Scab-infected nuts do not fill properly. Due to the close relationship between the amount of scab control on nuts and the weight of nuts from sprayed trees, the number of nuts per pound can be used as an index of the effectiveness of fungicide treatments (6, Table 4). The average number of nuts per pound for each treatment was determined at harvest during November 1960 by counting the number of nuts in 1-pound samples of representative nuts from each tree.

Table 3. Effectiveness of protectant fungicides for control of scab on Western pecan trees at Paden, Oklahoma during 1960.

Fungicide	Rate/100 gallons of spray	Application interval (in weeks)	Av. no. of nuts/lb (11/8/60)
Parzate	3 lb	2[a]	58
Cyprex	1 lb	do.	61
Dyrene	4 lb	do.	58
Parzate	3 lb	3[b]	63
Cyprex	1 lb	do.	64
Dyrene	4 lb	do.	67
Parzate + Plyac	3 lb + 2 oz	do.	61
Parzate + du Pont Spreader-Sticker	3 lb + 2 oz	do.	59
Parzate C + Esso LO-3328	3 lb + 1 qt	do.	62
Cyprex + Plyac	1 lb + 2 oz	do.	67
Cyprex + p. e. p. s.	1 lb + 8 oz	do.	65
Dyrene + Plyac	4 lb + 2 oz	do.	62
Dyrene + p. e. p. s	4 lb + 8 oz	do.	63
Dithane Z-78 + p. e. p. s.	3 lb + 8 oz	do.	62
None (Check)	---	---	90[c]

[a] Eight applications: 5/9, 5/23, 6/6, 6/20, 7/5, 7/18, 7/29, and 8/15.
[b] Five applications: 5/10, 5/31, 6/21, 7/12, and 7/29.
[c] Nuts incompletely filled and non-marketable.

Table 4. Effectiveness of protectant fungicides for control of scab on Squirrel pecan trees at Paden, Oklahoma during 1960.

Fungicide	Rate/100 gallons of spray	Application interval (in weeks)	Av. no. of nuts/lb (11/8/60)
Dithane Z-78	3 lb	2[a]	67
0-3818B	3 lb	do.	56
Dithane Z-78	3 lb	3[b]	60
Dithane Z-78 + National Sticker	3 lb + 1 qt	do.	60
Dithane Z-78 + Triton B-1956	3 lb + 2 oz	do.	57
Dithane Z-78 + Crag Glyodin	2 lb + 1 qt	do.	60
None (Check)	---	---	98[c]

[a] Eight applications: 5/9, 5/23, 6/6, 6/20, 7/5, 7/18, 7/29, and 8/15.
[b] Five applications: 5/9, 5/30, 6/20, 7/11, and 7/28.
[c] Nuts incompletely filled and non-marketable.

RESULTS AND CONCLUSIONS

All of the sprayed trees, regardless of treatment, had none to only trace amounts of scab on nuts and foliage in September. The unsprayed check trees were moderately to severely infected. The efficiency of the various fungicides, when used individually or with a spray adjuvant, is shown in Tables 3 and 4. Parzate, Dithane Z-78, Cyprex, Dyrene, and 0-3818B provided excellent and equivalent control of scab when applied on a 2-week schedule. Parzate, Cyprex, and Dyrene, when applied on a 3-week schedule, provided slightly less control. 0-3818B was not sprayed on a 3-week schedule.

The addition of adjuvants to the fungicide sprays did not provide control over that provided by the fungicides alone. This failure may be attributed to one or more of several factors: 1) The wetting action of some of the adjuvants may have caused excessive run-off of the fungicides;

2) The adjuvants may have reduced the activity of the fungicides; 3) The adjuvants did not retard weathering of the fungicides significantly; 4) Insufficient quantities of some of the adjuvants may have been used, and 5) Redistribution of fungicides over newly developed tissues may have been insufficient. Higher rates of the non-surfactant type adjuvants should be field tested in the future.

All of the tested fungicides provided excellent control. The zineb fungicides, Parzate and Dithane Z-78, are currently recommended for use in Oklahoma and Texas (2, 3, 12). Dyrene was also effective in the 1958 and 1959 tests in Oklahoma (1, 2), Rohm and Haas 0-3818B was effective in this test but it requires further testing. Dyrene has not been cleared for use on pecans. Cyprex was also effective in Oklahoma in 1960 and in Georgia (2, 4). Cyprex (dodine) is approved for use on pecans if it is not applied after shucks have started to split and with the limitation that meat or dairy animals cannot be allowed to graze in treated orchards. This recommendation will be made in Oklahoma for the 1962 season.

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SYSTEMIC INSECTICIDES FOR INDIRECT CONTROL OF BEAN YELLOW MOSAIC VIRUS IN SEED FIELDS OF YELLOW LUPINE[1]

D. B. Leuck[2], Homer D. Wells[3], and E. W. Beck[2]

Abstract

Biological assay studies and census data on aphid and bean yellow mosaic virus (BYMV) showed that dimethoate, phorate and O, O-diethyl S-2-(ethylthio) ethyl phosphorodithioate (Di-Syston) reduced populations of the aphid vectors through persistent systemic action and thereby reduced the secondary spread of BYMV in yellow lupine fields. Dimethoate was the most effective of the three insecticides.

INTRODUCTION

Between 1940 and 1950 yellow lupine (*Lupinus luteus*) was grown extensively as a green manure crop and as winter pasture in northern Florida and southern Georgia. At present this crop is not planted in these areas because of the adverse effects of bean yellow mosaic virus (BYMV) on seed production. This virus has been reported on yellow lupine by Corbett (2), who demonstrated that it is seed-borne and is also present in a wide range of annual and perennial hosts common in northern Florida and southern Georgia. The green peach aphid (*Myzus persicae* (Sulz.)) and the cowpea aphid (*Aphis medicaginis* Koch) are the most important vectors of the disease in the field according to Corbett (1). As a means of reducing incidence of the disease, Edwardson and Corbett (3) conducted experiments in which virus-free seed was planted in fields surrounded by a 24-foot border of oats, which they reported to be immune to the virus. Their experiments showed that lupine plantings surrounded by oat borders yielded significant increases in seed for two seed generations, but in the third seed generation all plots were 100% infected with BYMV by the time plants reached maturity.

In 1954 Benton, et al.[4] initiated a series of exploratory tests at Tifton, Georgia. In these tests several insecticides were evaluated for controlling possible aphid vectors and thereby reducing incidence of BYMV in yellow lupine. The tests were conducted on replicated plots and valuable information was obtained on the effectiveness of the insecticides against insects occurring on yellow lupine. However, in some years sufficiently heavy populations of vectors built up on the check plots to result in almost 100% virus infection throughout the experimental areas. Thus, in the fall of 1960 the authors conducted an experiment in which the more effective insecticides for the control of aphids, as determined by Benton, et al.[4], were evaluated for BYMV control in isolated plantings.

MATERIALS AND METHODS

On December 13, 1960 four fields, from 1/2 to 1 acre in size and isolated from each other by a distance of at least 400 yards, were uniformly planted in 36-inch rows at the rate of 20 pounds of yellow lupine seed per acre. The seed used had been harvested the previous year from an area heavily infected with BYMV (the seed had been indexed in an insect-proof screen house and approximately 7% carried virus). Phorate, O, O-diethyl S-2-(ethylthio)ethyl phosphorodithioate (Di-Syston), and dimethoate were applied at the rate of 2 pounds per acre of 10% active ingredient in granulated formulations. On January 31, 1961 the granules were applied in 8-inch bands over rows of seedlings 2 to 4 inches high. A second application of dimethoate was made on March 6 and of phorate and Di-Syston on March 13. Aphid populations were determined at intervals as shown in Table 1 by taking a 10-raceme sample from each field. The insects were counted after recovery in modified Berlese funnels. The residual effectiveness of the insecticides was compared on the dates given in Table 2, by means of biological assay with adult *Drosophila melanogaster* Meig., exposed for 48 hours to macerated material from four 10-gram raceme samples per field.

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[1] Cooperative investigations at Tifton, Georgia between the Entomology and Crops Research Divisions, Agricultural Research Service, United States Department of Agriculture and the University of Georgia, College of Agriculture Experiment Station, Georgia Coastal Plain Experiment Station. Published with the approval of the Director as Journal Series Paper No. 93.

[2] Entomologist, Entomology Research Division.

[3] Pathologist, Crops Research Division.

[4] Unpublished data.

On March 27, 1961 a 100-plant sample was staked off in each of 15 randomly selected rows in each of the test fields. The number of plants in each sample showing BYMV symptoms was recorded on May 8.

RESULTS

Population counts, summarized in Table 1, showed that aphids were present in numbers in the untreated field throughout the sampling period, and also that their average numbers were reduced by all of the insecticide treatments. The field treated with dimethoate had the lowest infestation throughout most of the sampling period as well as the lowest average infestation. The phorate treatment ranked second in reducing aphid populations. The field treated with Di-Syston had an average aphid population of more than three times that of the dimethoate-treated field and more than twice that of the phorate-treated field.

Comparative biological assays (Table 2) indicated that residues toxic to *Drosophila* were present in plants from fields treated with the three systemic insecticides. The residual effectiveness of dimethoate and phorate were about equal and greater than that of Di-Syston.

Counts of plants with disease symptoms (Table 3) showed that all insecticide treatments were highly effective in reducing the incidence of virus when compared with those taken in the untreated field, which had a high incidence of virus. The field treated with dimethoate had the lowest number of virus-infected plants. Phorate was more effective than Di-Syston in reducing the incidence of virus.

Table 1. Effectiveness of granular systemic insecticides applied January 31 and March 13[a] at 2 pounds per acre on populations of aphids on yellow lupine.

Insecticide	Aphid counts per 10-raceme sample on indicated dates								
	February 27	March 6	March 22	March 26	April 3	April 10	April 17	April 24	Average
Phorate	3	5	20	5	15	15	3	13	10
Di-Syston	3	12	10	0	35	5	18	90	22
Dimethoate	0	0	0	0	0	3	43	3	6
Control	60	80	10	40	45	53	20	33	43

[a] Second application of dimethoate applied March 6.

Table 2. Comparative residual effectiveness of granular systemic insecticides applied January 31 and March 13[a] at 2 pounds per acre as indicated by bioassay tests with *Drosophila melanogaster* adults (48 hours of exposure to 10 grams of macerated yellow lupine racemes).

Insecticide	% mortality of fruit flies on indicated dates			
	February 28	March 6	March 24	April 17
Phorate	100	97	100	5
Di-Syston	59	45	74	4
Dimethoate	99	87	100	9
Control	9	13	8	7

[a] Second application of dimethoate applied March 6.

Table 3. Effect of systemic insecticides applied January 31 and March 13[a] at 2 pounds per acre on incidence of BYMV in yellow lupine. (Determined from virus counts of 1500 plants per field after seed set on May 8.

Insecticide	% of plants showing BYMV symptoms
Phorate	23
Di-Syston	33
Dimethoate	13
Control	81

[a]Second application of dimethoate applied March 6.

DISCUSSION

Bioassay and aphid counts indicated that the systemic insecticides used in this test furnished effective aphid control for about 4 weeks after application in February or March. These data indicated that an additional application might have been desirable from the standpoint of controlling virus vectors. However, the buildup of aphid populations in some treated fields after the effectiveness of the insecticides disappeared may not have been reflected in increased incidence of virus due to the advanced stage of maturity of the plants at that time.

The aphid and virus census data from the control field showed that BYMV vectors were present in the Tifton area and a high incidence of disease resulted when a seed field was planted with seed of which about 7% was infected with virus. Since the experimental fields were not replicated and data were obtained from only 1 year's study, definite conclusions cannot be drawn on the comparative merits of the different treatments. However, all systemic insecticide treatments reduced the incidence of virus below that encountered in the check and that observed in previous experimental plantings at this station and on surrounding farms during the past 7 years. The reduction in virus incidence from 81% in the control to 13% for plants receiving two applications of dimethoate (Table 3) suggested that this chemical has potential for controlling BYMV vectors and virus buildup in commercial yellow lupine seed fields. Data indicated that secondary spread of BYMV within a field can be materially reduced through the use of systemic insecticides, without the use of immune oats as reported by Edwardson and Corbett (3).

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COMPARISON OF FLOWERING CRAB APPLE VARIETIES FOR FAST DETECTION OF A COMMON LATENT VIRUS IN APPLES[1]

J. E. Reynolds and J. A. Milbrath[2]

Abstract

The reactions of 16 varieties of flowering crab apples to inoculation with a latent apple virus were studied. A rapid reaction was obtained in the greenhouse by whip-and-tongue grafting the crab apple varieties on apple seedlings and chip-budding the inoculum below the graft. Hopa was selected as the best index host variety because it reacted to and differentiated mild or severe strains of virus. Preliminary experiments suggested the virus involved to be the one responsible for chlorotic leaf spot and stem pitting of apples.

INTRODUCTION

Apple chlorotic leaf spot (ACLS) was the name proposed by Mink and Shay for a virus latent in many apple varieties. The comprehensive review of the apple virus problem by McCrum, et al. (3) indicates that this virus is widespread and may be involved in stem pit and many of the symptoms described for some other apple virus diseases. Mink and Shay (2) tested several Russian apple seedlings as indicators for ACLS and the stem pitting virus and Blodgett and Aichele (1) used flowering crabs to detect stem pit virus. Reynolds and Milbrath (4) reported the effect of latent apple viruses on 99 varieties of flowering crab[3]. Further study of the 12 varieties which reacted most significantly gave the results presented in this report.

METHODS AND MATERIALS

The 12 varieties and species of flowering crab which had given the most significant reaction in previous studies were: Malus theifera, M. sargenti, M. atrosanguinea, Purple Lemoine, Ming Shing, Exzellenz Thiel, Jay Darling, Purple Wave, Crimson Brilliant, Profusion, Hopa, and P.I. 78034. The varieties Eleyi and Van Eseltine were reported by Blodgett and Aichele (1) to be strong reactors. Although these varieties had given no reaction in preliminary studies by Reynolds and Milbrath (4) the OSU clones were included for retesting. The Russian seedling R127407A obtained from Mink and Shay was also used in these comparisons.

The inoculum for these studies was obtained from five sources of apples that had previously given five categories of reaction ratings ranking from 0 to 4 when tested on Hopa.

Each test variety was bench-grafted on 15 apple seedlings with a 2-bud whip-and-tongue graft placed 6 to 8 inches above the crown of the seedlings. The trees were then divided into five groups of three trees each and each group was inoculated with two chip buds from apple sources which previous Hopa indexing had given virus reaction ratings classified from 0 to 4. The chip buds were placed on opposite sides of the stem between the top graft and the crown. Parafilm M cut to 1/2-inch spools on a band saw was used to cover the cut surfaces of the graft and the chip buds. The cut tip of the scion was sealed with hot grafting wax.

The trees were planted in #10 tin cans and then set on ground beds in a greenhouse where the air temperature was approximately 70°F during the test interval, February 1 to April 13. Virus symptoms became apparent soon after new growth developed.

RESULTS

Jay Darling, M. theifera, Purple Wave, Purple Lemoine, Ming Shing, and Crimson Brilliant reacted rapidly and severely to all sources of inoculum. Even when inoculated with sources of apple which had given mild virus reactions on Hopa, these grafts produced very little growth. Only the trees inoculated with a Hopa negative source developed normal growth (Fig. 1A).

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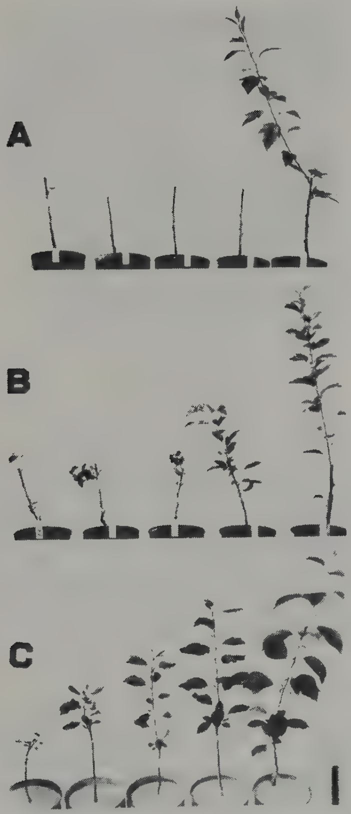
[1] Technical Paper No. 1509, Oregon Agricultural Experiment Station. Taken in part from Ph. D. thesis data of senior author.

[2] Graduate Assistant and Professor, respectively, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

[3] The flowering crab apple varieties used in this study were from the OSU collection, Horticultural Farm, Corvallis.

FIGURE 1. Typical responses of flowering crab varieties to latent apple viruses. Each tree represents one of a series of three trees inoculated with a Hopa 0, 1, 2, 3 and 4, from right to left. All of the varieties were inoculated with the same source of virus. A -- Purple Lemoine illustrates the very severe reaction. B -- M. sargenti shows less severe reactions but not uniformity in severity. C -- Hopa showing the gradual increase of severity from normal on right to very severe on left.

FIGURE 2. M. theifera illustrating the type reactions when severe reacting varieties were inoculated 7, 14, 21 and 28 days after grafting with a Hopa 4 type virus.



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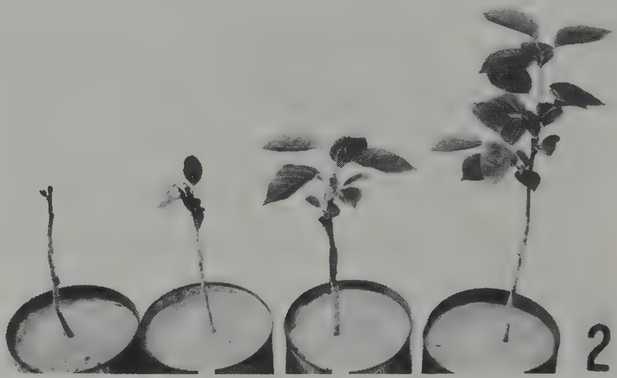

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Seven other varieties showed a graduation of symptom severity which varied from a mild chlorotic or red spotting of leaves to a severe mottling rugosity and cupping of leaves. The new growth of these plants also showed varying degrees of elongation (Fig. 1, B, C). In all, seven varieties showed some variation in virus severity, but the Hopa variety was more uniform in its reaction to virus strains (Table 1).

Table 1. Reaction of varieties of flowering crab apples to five sources of virus selected on basis of Hopa indexing.

Variety	Degree of severity of inoculum sources				
	Hopa 4	Hopa 3	Hopa 2	Hopa 1	Hopa 0
Jay Darling	4	4	4	4	0
M. theifera	4	4	4	3	0
Purple Wave	4	4	4	4	0
Purple Lemoine	4	4	4	4	0
Crimson Brilliant	4	4	4	4	0
Ming Shing	4	4	4	4	0
Profusion	2	3	1	1	0
P.I. 78034	4	3	3	1	0
M. atrosanguinea	2	2	2	2	0
Exzellenz Thiel	2	2	1	1	0
M. sargenti	3	3	3	2	0
Hopa	4	3	2	1	0
Russian seedling	-	-	-	-	-
M. eleyi	0	0	0	0	0
Van Eseltine	0	0	0	0	0

Eleyi and Van Eseltine failed again to give any reaction to these inoculations. This would indicate the clones of these two varieties used by Blodgett and Aichele (1) differ from the OSU clones used in this study.

No data were included in Table 1 for the Russian seedling since the results with this variety were unsatisfactory. In each of the groups of three trees, there were normal appearing plants and dead grafts. The dead grafts were assumed to be due to graft failure rather than virus reaction since there were no apparent differences between the various inoculations. Even the plants that grew did not show the mottling and distortion found in the other varieties.

The data in Table 1 show that several varieties of flowering crab could be used in indexing for virus freedom. The Hopa variety has been used in Oregon for a winter indexing program and has proven to be very reliable in detecting virus as well as indicating the severity of the strain of virus involved. The more severely reacting varieties of crab gave no indication of strain of virus involved, or whether lack of growth was due to a virus or to graft failure.

The effect of time of inoculation was tested by inoculating five severely acting varieties 7, 14, 21 and 28 days after the test variety had been grafted and planted. The crab varieties Jay Darling, M. theifera, Purple Wave, Purple Lemoine, and Crimson Brilliant were used in this experiment. The source of inoculum was an apple variety which had given a virus reaction of 4 on Hopa. Figure 2 shows the general effect of delay in inoculation.

Inoculation 7 days after grafting still caused a virus reaction that was too severe to allow much or any growth. However, the later inoculations became more comparable to the Hopa reaction, with the severity becoming less and less as the interval increased between grafting and inoculation. This experiment demonstrated the importance of time of inoculation to subsequent virus reactions.

DISCUSSION

Extensive index tests in Oregon with Hopa crab showed this to be a valuable index host for latent apple viruses. Under greenhouse conditions, the reactions were rapid and reliable. Hopa had the advantage over the more severely reacting varieties in that it detected degrees of virus severity, and allowed the selection of apple sources which graded from no reaction to very severe reaction. These sources could be used as a basis for study of the complex of latent viruses that exist in apple (4).

The virus or viruses producing the graded reactions in Hopa, and probably in the other varieties, could be the virus or virus strains responsible for chlorotic leaf spot or stem pitting. Preliminary experiments support the theory that the Hopa reaction may be caused by strains of the same virus. An apple source which gave no reaction on Hopa failed to cause any decline of Spy 227 or stem-pitting of Virginia crab, while an apple source with a Hopa 4 virus reaction gave a severe decline of Spy 227 and a severe stem pitting reaction on Virginia crab. The apple sources which resulted in milder reactions on Hopa also produced milder effects on Spy 227 and on Virginia crab. These observations make the Hopa crab important as an index host and as an experimental tool.

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CHLOROPICRIN INJURY ON SWEETPOTATOES[1]

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In late February 1961, an undescribed disorder of sweetpotato was reported from a commercial storage-house in southern New Jersey. Superficial, sunken, circular lesions, up to 1 cm in diameter were observed on the Jersey Orange and Jersey Red varieties. Similar but horizontally elongate lesions were observed on the White Yam variety. In both cases the lesions were found principally at the buds. Attempts to isolate a pathogen failed.

Chloropicrin was suspected when it was discovered that an adjacent, empty storage-house had been fumigated for rodent control, and that a greater degree of injury was noted on potatoes at the floor level.

PROCEDURE

Sound Jersey Red, Jersey Orange, and White Yam sweetpotatoes, stored over winter, were fumigated with chloropicrin at 2, 5, 25, 125, and 250 ppm for 24 hours and with 500, 1000, 2500, and 5000 ppm for 1/2 hour. Data were taken on 2 replicates of 10 potatoes each after 4 days for the higher-dosage treatments, and after 6 and 12 days for the lower-dosage treatments.

A similar experiment was performed with sweetpotatoes stored for approximately 3 weeks after harvest. Two additional varieties, Yellow Jersey and Virginian, were included in this test.

RESULTS

Lesions identical to those of the commercially stored potatoes were produced on the test potatoes by chloropicrin (Figures 1 and 2). Potatoes stored for a relatively short time after harvest were considerably more susceptible to chloropicrin than those stored over winter (Tables 1 and 2). As little as 2 ppm chloropicrin produced visible lesions between 6 and 12 days after exposure to the gas on those varieties previously stored for a short time after harvest.

Table 1. Effects of various dosages of chloropicrin on three sweetpotato varieties stored over winter.

Dosage (ppm)	Jersey Orange % injured	Jersey Orange Av. spots/ potato	White Yam % injured	White Yam Av. spots/ potato	Jersey Red % injured	Jersey Red Av. spots/ potato
	1/2-hour Fumigation-Evaluation after 4 days					
5000	100	21	100	15	93	3
2500	100	17	100	6	33	1
1000	100	10	54	2	0	
500	100	6	0		0	
	24-hour Fumigation-Evaluation after 6 days					
250	100	33	100	35	90	3
125	100	11	0		0	
25	0		0		0	
5	0		0		0	
2	0		0		0	
	24-hour Fumigation-Evaluation after 12 days					
250	100	50	100	48	100	20
125	100	18	100	10	90	4
25	100	14	0		90	3
5	0		0		0	
2	0		0		0	

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers - the State University, New Brunswick, New Jersey.

Table 2. Effects of various dosages of chloropicrin on five sweetpotato varieties stored for approximately 3 weeks after harvest.

Dosage (ppm)	Jersey Orange % injured	Jersey Orange Av. spots/ potato	White Yam % injured	White Yam Av. spots/ potato	Jersey Red % injured	Jersey Red Av. spots/ potato	Yellow Jersey % injured	Yellow Jersey Av. spots/ potato	Virginian % injured	Virginian Av. spots/ potato
1/2-hour Fumigation-Evaluation after 4 days										
5000	100	49	100	53	100	11	100	14	100	37
2500	100	31	100	26	100	7	100	13	100	22
1000	100	14	70	5	60	4	60	3	60	3
500	100	8	20	1	20	1	40	2	20	6
24-hour Fumigation-Evaluation after 6 days										
250	100	63	100	59	100	17	100	29	100	36
125	100	28	100	28	100	9	100	13	90	28
25	80	5	50	4	40	4	30	2	40	3
5	0		0		0		0		0	
2	0		0		0		0		0	
24-hour Fumigation-Evaluation after 12 days										
250	100	75	100	63	100	30	100	32	100	45
125	100	38	100	32	100	12	100	17	100	32
25	100	9	50	5	100	5	90	4	100	4
5	100	5	0		100	4	50	4	80	4
2	70	4	0		50	3	0		40	4

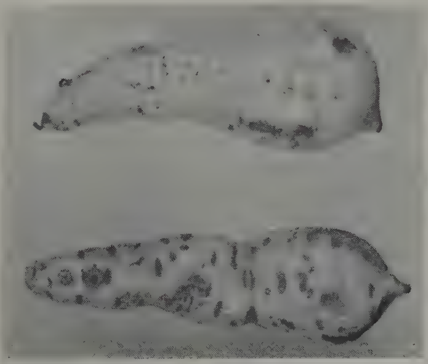

FIGURE 1. Right -- Chloropicrin injury on the White Yam variety. Left -- Sound Potato.

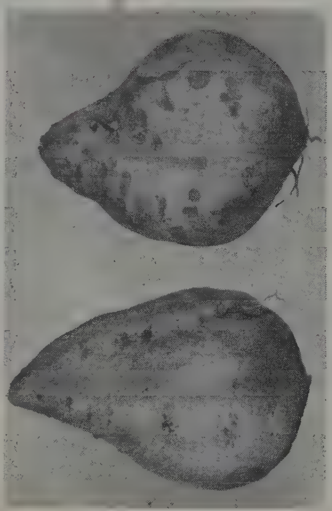

FIGURE 2. Left -- Chloropicrin injury on the Jersey Orange variety. Right -- Sound potato.

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A SECOND PATHOGENIC RACE OF PSEUDOMONAS SESAMI

C. A. Thomas, R. G. Orellana, M. L. Kinman, and G. W. Rivers

Summary

Susceptibility of the sesame variety Margo to bacterial leaf spot in recent years was found to be due to a new pathogenic race of Pseudomonas sesami. Isolates studied, alike morphologically and physiologically in several tests, differed markedly in pathogenicity to Margo. Other varieties tested were either equally susceptible or resistant to the two races.

Sesame, Sesamum indicum, is an ancient oilseed plant that is grown in the United States primarily for whole seed used by the bakery trade. Bacterial leaf spot, incited by Pseudomonas sesami Malkoff, has been reported from most areas where sesame is grown. This disease, noted in experimental or commercial plantings in the United States since 1943 (1), currently is serious in commercial fields in Texas. A similar leaf spot, incited by Xanthomonas sesami Sabet & Dows., occurs in Africa (2).

Two sesame breeding lines, Early Russian and B-1, were identified as sources of resistance in 1952 and 1953, respectively. Selections from B-1 proved highly resistant in many tests in the United States during 1953-1957. In these same tests, the varieties Venezuela 51-6, Delco and many others were highly susceptible. In 1957, the varieties Margo (a B-1 selection, B-1-8-6) and Dulce (a resistant selection from the cross Rio x B-1-8) were released for commercial production; by 1959 these two varieties occupied 90% of the commercial acreage in the United States. In 1958, Margo and Dulce were susceptible in commercial plantings in west Texas. Margo and all of its previously resistant relatives were severely damaged in experimental plantings at Muleshoe, Texas; inoculum to initiate this epiphytotic originated in an adjacent area planted to commercial Margo seed. It is not known whether the original source of this seed-borne inoculum was seed from commercial seed fields grown in 1957 or foreign seed cleaned with the equipment later used to clean planting seed. Since 1958, Margo and its derivatives have been susceptible wherever the new strain of P. sesami was present (Table 1). Field damage to Margo was never quite as severe as to Delco and Venezuela 51. Early Russian selections have remained resistant at all locations, but traces of infection have been present at Muleshoe, Texas since 1958 and were found at Lubbock, Texas in 1961.

Table 1. Reaction[a] of certain sesame varieties to Pseudomonas sesami in Texas[b], 1953-61.

Year	Delco (SI 77)	Venezuela 51 (SI 6)	Margo (SI 39)	Early Russian (SI 32)
1953	S	S	R	R
1954	S	S	R	R
1955	S	S	R	R
1956	S	S	R	R
1957[c]	-	-	-	-
1958	S	S	S	R
1959	S	S	S	-[c]
1960	S	S	S	R
1961	S	S	S	R

[a] S-susceptible; R-resistant.
[b] Tests at College Station 1953-56, Lubbock 1961, and Muleshoe 1958-61.
[c] No data available.

In 1959, 12 isolates were obtained from naturally infected Margo plants at Muleshoe, Texas. Thirty-three isolates were obtained from naturally infected Venezuela 51 plants at Beltsville, Maryland, in a test in which Margo was resistant. These isolates were used to inoculate 8-week-old plants of the varieties Delco, Venezuela 51, Margo, and Early Russian in the greenhouse under nearly optimum conditions of sunlight. All varieties except Early Russian were susceptible to the Margo isolates whereas only Venezuela 51 and Delco were susceptible to the isolates from Venezuela 51.

The original two groups of isolates were reisolated from the greenhouse plants and subsequently further purified by transferring single colonies from successive dilution plates. Inoculations with these cultures in the greenhouse, at times under sub-optimum light conditions, gave erratic results. Light intensity, day length, and nitrogen fertilization appeared to affect varietal reaction.

Consistent and repeatable results were obtained through the use of growth-room facilities. Venezuela 51, Margo, and Early Russian were grown in a composted soil-vermiculite mixture on a 16-hour day, 1500 foot-candles, at a day temperature of 30° C and a night temperature of 20°. Plants were fertilized 2 weeks after planting with NH_4NO_3. Six-week-old plants were inoculated by gently atomizing leaves with bacteria from 48-hour-old nutrient-agar cultures suspended in distilled water. Plants were in a moist chamber 16 hours before inoculation.

In four complete and separate tests, each consisting of 16 plants of each variety inoculated with a Margo isolate and a similar set of plants inoculated with a Venezuela 51 isolate, Venezuela 51 was highly susceptible to both isolates (Fig. 1). No difference in pathogenicity or virulence was noted. Margo, however, was susceptible to the Margo isolate and resistant to the Venezuela 51 isolate (Fig. 2). Early Russian was resistant to both.

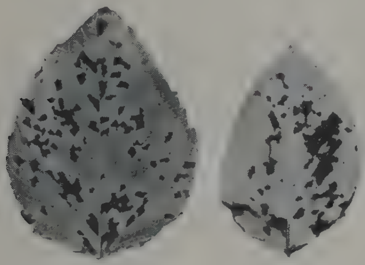

FIGURE 1. Reaction of Venezuela 51 sesame to Margo isolate (left) of P. sesami and Venezuela 51 isolate.

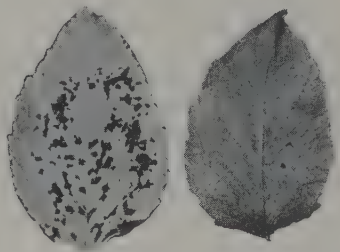

FIGURE 2. Reaction of Margo sesame to Margo isolate (left) of P. sesami and Venezuela 51 isolate.

Table 2. Morphology and physiological characteristics of isolates of Pseudomonas sesami.

Character	Margo isolate[a]	Venezuela 51 isolate[a]
Flagella	2-5 polar	2-5 polar
Shape	straight rod	straight rod
Acid from glucose	+	+
sucrose	-	-
lactose	-	-
starch	-	-
Nitrites from nitrates	-	-
Indol production	-	-
H_2S production	-	-
Pigment	-	-
Gram stain	-	-
Gelatin liquefaction	+	+
Milk turned alkaline	+	+
Urea utilized	+	+
5% NaCl tolerance	+	+
7% NaCl tolerance	-	-
Asparagine sufficient as only source of N and C	+	+

[a] Two or more isolates were used in each test. Tests were run two or more times.

Margo and Venezuela 51 isolates were compared morphologically and physiologically by procedures given by Stapp (3). The results (Table 2) indicated that the isolates were similar. The only differences noted were that certain Margo isolates liquefied gelatin and turned milk alkaline somewhat faster than the Venezuela 51 isolates did.

The results of the tests described indicate that a new pathogenic race, which may be designated Race 2, of *P. sesami* is responsible for the present susceptibility of the variety Margo. The development and occurrence of this new race after the release and commercial production of a resistant variety are similar to that of Race 2 of *Xanthomonas malvacearum* (E. F. Sm.) Dows. The results of other physiological and biochemical tests relating to the pathogenicity of the two races as well as to the differences in varietal reaction can be noted elsewhere (4).

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CONTROL OF BACTERIAL SPOT OF PEACH WITH DODINE[1]

Robert H. Daines[2]

Summary

In an experiment where 22 treatments were tested for control of bacterial spot, dodine was the most effective bacteriocide used. A dodine-sulfur mixture was highly significantly more effective in the control of this disease than dodine or a dodine-captan mixture. The dodine-sulfur combination also markedly increased the amount of injury (yellow spots) developing on the fruit. Captan, when added to dodine, did not improve bacterial spot control nor did it safen dodine, as measured by the occurrence of yellow spots on the fruit. Dodine 1 pound + sulfur 3 pounds, and dodine 1 pound + captan 1 pound provided significantly better protection against this disease than did zinc sulfate-lime. Dodine at the 1/2 pound rate, alone or with sulfur or captan provided protection equal to the zinc sulfate-lime or captan at 4 pounds. Vancomycin, EP 166 phenacridane chloride (9-(p-n-hexyloxyphenyl)-10-methyl-acridinium chloride), and manganese Nabac (tank mixed using sodium Nabac and manganese sulfate), as used provided some protection against bacterial spot.

In 1960, in New Jersey dodine provided the best protection (1) against bacterial spot of peach caused by Xanthomonas pruni (E. F. Sm.) Dows. In 1961 this material was used at 1/4, 1/2, and 1 pound levels, alone, and in combination with 1 pound of captan or 3 pounds of sulfur. Several other compounds were also tested (Table 1). All materials were applied on a 10-day to 2-week schedule, beginning with the shuck-split application and continuing until harvest. The sprays were applied from the ground to five randomized, uniform-size, 6-year-old Sunhigh peach trees, using an eight-cluster nozzel gun at 300 pounds' pressure.

In the experiment reported here dodine, as in 1960, provided more protection against bacterial spot than any of the other materials used (Table 1). The addition of captan to the dodine sprays did not significantly affect protection at any of the concentrations used, but this was not so when sulfur was added. The most effective treatment was dodine at the 1-pound level with 3 pounds of sulfur. This combination was significantly better at the 1% level than dodine at 1 pound alone or with the addition of captan. And, again, the effectiveness of dodine at 1/4 pound was increased significantly (5% level) when sulfur was added. It is very encouraging that, for the second year in a row, certain dodine treatments were significantly better than the standard zinc sulfate-lime treatment. Control with dodine at 1 pound with sulfur was highly significant and with captan the protection over that offered by zinc sulfate-lime was significant. The antibiotic Vancomycin, phenacridane chloride (9-(p-n-hexyloxyphenyl)-10-methyl-acridinium chloride) (EP 166), and manganese Nabac[3] (tank mixed), at the concentrations used, provided some protection against bacterial spot, although the ripening of the fruit on trees sprayed with Nabac was delayed slightly and exhibited some fruit russeting.

The experiment reported by Diener and Carlton (3) involved too many variables to allow conclusions concerning the effects of combinations of dodine and other spray materials on foliage injury. Their three treatments that showed injury had dodine and malathion in common, and the data suggest that this combination might be at fault. Leaf spotting on peaches following the use of dodine has not been observed in New Jersey. In fact, in some instances dodine-sprayed trees were outstanding in respect to the excellence of their foliage. Although leaf drop from bacterial-spot infections did occur on the dodine-sprayed trees, the amount of leaf fall was somewhat less than that of trees sprayed with other materials. While dodine provided some protection against leaf infections, it was not so great as that occurring on the fruit.

Fruit spotting on dodine-sprayed trees as reported in 1960 (1) was again evident on some of the treatments (Fig. 1). The fruit injury was first observed at about the stone-hardening period as a slight retardation in size and an increase in the yellow ground color. As the fruit matured the red areas exhibited small yellow spots. On severely affected fruits maturity was delayed 2 to 3 days. The great increase in fruit injury wherever sulfur and dodine were used

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[3] Nabac contains 25% hexachlorophene WP.

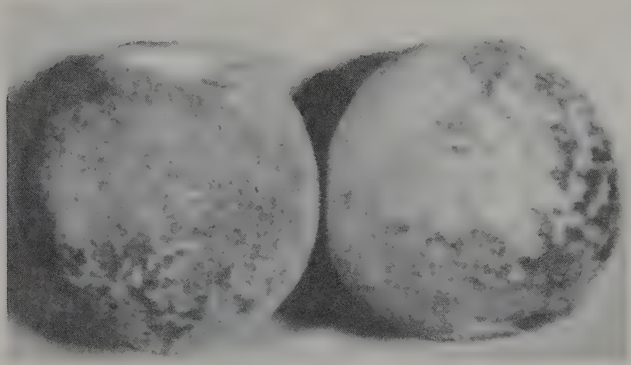

FIGURE 1. Yellow spotting and blotching of peach fruits sprayed with dodine and sulfur.

Table 1. Incidence of bacterial spot on peach fruits sprayed with various fungicides.

Treatment, unless otherwise indicated, concentration in lb/100 gal.[a]	% fruits affected[b]	Level of significance	
		5%[c]	1%[d]
Dodine 1, sulfur 3	2.4	A	1
Dodine 1, captan 1	5.3	B	2
Dodine 1	6.1	B, C	2, 3
Dodine 1/2, sulfur 3	7.0	B, C, D	2, 3, 4
Dodine 3/4	7.9	B, C, D	2, 3, 4
Dodine 1/2, captan 1	8.0	B, C, D, E	2, 3, 4
Dodine 1/2	8.3	C, D, E	2, 3, 4, 5
Zinc sulfate (basic) 2 1/2, lime 6, sulfur 6	8.4	C, D, E	2, 3, 4, 5
Dodine 1/2, glycerin 1 gal.	8.5	C, D, E	2, 3, 4, 5
Dodine 1/4, sulfur 3	9.9	D, E, F	3, 4, 5, 6
Dodine 1/4, captan 1	11.2	E, F, G	4, 5, 6, 7
Captan 4	12.5	F, G, H	5, 6, 7, 8
Dodine 1/4	14.5	G, H, I	6, 7, 8, 9
Vancomycin 100 ppm	15.8	H, I	7, 8, 9, 10
EP 166, 200 ppm	16.9	I, J	8, 9, 10, 11
EP 166, 400 ppm	17.9	I, J	9, 10, 11
Sodium Nabac, tank mixed[e]	20.2	J, K	10, 11
Captan 3	22.6	K, L	11, 12
EP 166, 100 ppm	22.6	K, L	11, 12
Sulfur 6	26.6	L, M	12, 13
Check, unsprayed	31.1	M, N	13, 14
Captan 2	34.5	N	14

[a] Sprays applied on a 10-day to 2-week schedule beginning with the shuck split. Parathion was used in all treatments at the shuck split, first, second, and fifth cover sprays.

[b] Percentages converted to angles for calculations. Angle = $\arcsin \sqrt{\text{percentage}}$

[c] Treatments followed by the same letter do not differ at the 5% level of significance, and

[d] Treatments followed by the same number do not differ at the 1% level of significance according to Duncan's multiple range test.

[e] Tank mixed sodium Nabac prepared (early sprays) by mixing in a bucket containing some water, 3 quarts of sodium Nabac and 171 grams of manganese sulfate. For later sprays 2 quarts of sodium Nabac and 114 grams of $MnSO_4$ were used.

together was most outstanding. For example, where 1 pound of dodine was used alone or with captan, spotting occurred on less than 5% of the fruit. Where 3 pounds of sulfur was added to dodine at 1, 1/2, and 1/4 pound, 90, 80, and 30% of the peaches showed this spotting, respectively. Sulfur alone had no such effect on the fruits.

The data from this experiment indicate that sulfur increases the value of dodine in disease control and also increases its phytotoxic properties. To achieve improved bacterial spot control by the use of chemical sprays, it has been postulated (1, 2) that increased absorption is necessary. Also, to destroy red pigment precursors resulting in the yellow spots would require penetration into the cells developing pigmentation. It appears likely, therefore, that a compound formed between dodine and a decomposition product of sulfur might be involved, and that such a compound would be absorbed more readily than dodine.

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DEPARTMENT OF PLANT PATHOLOGY, NEW JERSEY AGRICULTURAL EXPERIMENT STATION, NEW BRUNSWICK

SPROUT DIP TREATMENTS FOR CONTROL OF BLACK ROT OF SWEETPOTATOES[1]

Robert H. Daines[2]

Summary

Experiments were conducted in which sweetpotato sprouts inoculated with Ceratocystis fimbriata were dipped in various fungicides in order to determine which were most effective against this pathogen. Coromerc (N-phenylmercuri-ethylene diamine 10%), 1 pint in 30 gallons, Semesan Bel (2-chloro-4-(hydroxy-mercuri) phenyl and isomers 3.2%; 4-(hydroxymercuri)-2-nitrophenyl and isomers, 12%), 1 pound in 10 gallons, and Coromerc 1 pint in 45 gallons, proved to be the most effective dip treatments in reducing black rot incidence in the crop at harvest time. Semesan Bel (1-10) and Coromerc (1 pint in 45 gallons) produced the best yields of salable sweetpotatoes. These materials used as sprout treatments out-performed Tersan OM (thiram 45% + hydroxymercurichlorophenol 10%), Dyrene (2,4-dichloro-6-(o-chloroanilino)-s-triazine), or captan 50% in the control of black rot and the production of salable sweetpotatoes.

The use of apparently disease-free sweetpotato bedding stock treated with seed and sprout fungicide dips has long been the standard method of controlling black rot, caused by Ceratocystis fimbriata Ell. & Halst., and other fungus diseases of the sweetpotato. Mercurials, borax, ferbam, chloranil, Tersan, captan, and other materials have been found sufficiently active against the various diseases to warrant commercial usage. New Jersey growers hesitate to use mercurials for sweetpotato seed or sprout dips since their use in our light soils has been followed by plant injury.

The literature lacks information on the relative merits of the newer fungicides or a comparison of them with the long-accepted fungicides. As early as 1947 and 1951 Jeffers (4) and Jeffers and Moore (5) reported that phenyl mercuries were effective against black rot, but they included little information on field performance. Daines (1, 2) and Daines, et al. (3) have already reported the effects of a number of these fungicides as seed potato dips on the control of black rot, Java black rot caused by Diplodia tubericola (Ell. & Ev.)Taub., and dry rot caused by Diaporthe batatatis (Harter & Field). In each case the mercurials proved most effective against the disease.

To determine the effect of various fungicidal dips on yields and on the control of black rot affecting the crop at harvest time, the following experiment was conducted.

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EXPERIMENTAL METHODS

For 4 consecutive years, beginning in 1958, Jersey Orange sweetpotato sprouts, taken from a source believed to be free of black rot, were pulled and rinsed in running water. The roots were dipped in a black rot spore suspension and the plants wrapped in burlap bags and placed in baskets, where they were held for 15 hours in a room maintained at 75° F and 90% relative humidity. After this infection period, the sprouts were dealt into nine piles and each pile treated as indicated in Table 1. After drying, the sprouts were wrapped in moist burlap and taken to the field and machine-planted in four, 9-row, 125 foot tiers, with each treatment randomized once in each tier. At harvest time 100-foot sections were harvested from each treatment in each replication. At that time, the sweets were divided into first- and second-size roots, but for convenience in this report all roots of salable size are grouped together. The sweets showing black rot and those that appeared to be free from this disease were counted and weighed.

Table 1. The effect of sprout dip treatments on black rot development in the resulting crop.

Fungicide dip treatment	% harvested sweets showing black rot[a]	Level of significance 5%[b]	1%[c]
Coromerc, 1 pt in 30 gal.	2.4	a	1
Semesan Bel, 1 lb in 10 gal.	3.9	a, b	1, 2
Coromerc, 1 pt in 45 gal.	6.4	b, c	1, 2, 3
Semesan Bel, 1 lb in 15 gal.	8.3	c, d	2, 3, 4
Captan, 1 lb in 5 gal.	11.3	d, e	3, 4, 5
Tersan OM, 1 lb in 5 gal.	12.4	e	4, 5
Coromerc, 1 pt in 60 gal.	13.1	e	4, 5
Dyrene, 1 lb in 5 gal.	14.4	e	5
Check, no dip treatment	25.9	f	6

[a] Data secured over a 4-year period with years serving as replicates in final calculations. Percentages converted to angles for calculations. Angle = arcsin $\sqrt{\text{percentage}}$

[b] Treatments followed by the same letter do not differ at the 5% level of significance, and

[c] Treatments followed by the same number do not differ at the 1% level of significance according to Duncan's multiple range test.

RESULTS

The data in Table 1 show that the mercurials were more effective against black rot than the nonmercurial fungicides. Also, Coromerc (N-phenylmercuriethylene diamine 10%), 1 pint in 30 gallons of water, provided protection against this disease that was statistically significant at the 1% level over all treatments used except Semesan Bel (2-chloro-4-(hydroxymercuri)phenyl and isomers 3.2%; 4-(hydroxymercuri)-2-nitrophenyl and isomers, 12%), 1 pound in 10 gallons and Coromerc, 1 pint in 45 gallons. It was noted, however, that the Coromerc, 1 pint to 30 gallons treatment, retarded sprout growth for 1 to 2 weeks after the sprouts were set in the field. That the yield for Coromerc 1-30 (Table 2) was lowest is interesting. Although the total yields between treatments showed no significant differences, Coromerc 1-30 was shown to just miss a significant yield reduction. Coromerc 1-30 produced significantly less yield of marketable sweetpotatoes than Coromerc at 1 pint in 60 gallons of water (Table 3).

As has already been observed, Coromerc at 1-45 and Semesan Bel 1-10 (Table 1) provided protection against black rot that was equal to that offered by Coromerc 1-30 at the 1% level of significance, and Table 3 indicates that Coromerc 1-45 and Semesan Bel 1-10 produced more salable pounds of sweetpotatoes than the Coromerc 1-30 treatment.

The Coromerc and Semesan Bel treatments generally out-performed captan 50%, Tersan OM (thiram 45% +hydroxymercurichlorophenol 10%), and Dyrene (2,4-dichloro-6-(o-chloroanilino)-s-triazine) in the control of black rot and in the production of apparently disease-free marketable sweetpotatoes.

Table 2. The effect of sprout dip treatments on the total yields of salable sized sweetpotatoes.

Fungicide dip treatment	Average total yields in 100 lb/acre of 1st and 2nd sized sweetpotatoes[a]
Coromerc, 1 pt in 60 gal.	230.3
Tersan OM, 1 lb in 5 gal.	224.5
Semesan Bel, 1 lb in 10 gal.	222.0
Check, untreated	220.5
Semesan Bel, 1 lb in 15 gal.	220.4
Captan, 1 lb in 5 gal.	218.7
Dyrene, 1 lb in 5 gal.	218.7
Coromerc, 1 pt in 45 gal.	217.9
Coromerc, 1 pt in 30 gal.	209.9

[a] Data secured over a 4-year period with years serving as replicates. No statistical significance exists between these treatments.

Table 3. The effect of sprout dip treatments on the yields of salable sized sweetpotatoes that appeared to be free from black rot at harvest time.

Fungicide dip treatment	Average yields in 100 lb/acre[a]	Level of significance 5%[b]	1%[c]
Coromerc, 1 pt in 60 gal.	212.8	a	1
Semesan Bel, 1 lb in 10 gal.	204.2	a, b	1, 2
Coromerc, 1 pt in 45 gal.	203.1	a, b	1, 2
Semesan Bel, 1 lb in 15 gal.	193.0	a, b, c	1, 2
Coromerc, 1 pt in 30 gal.	189.4	b, c	1, 2, 3
Tersan OM, 1 lb in 5 gal.	186.6	b, c, d	1, 2, 3
Dyrene, 1 lb in 5 gal.	181.9	b, c, d	1, 2, 3
Captan, 1 lb in 5 gal.	177.9	c, d	2, 3
Check, untreated	156.4	d	3

[a] Data secured over a 4-year period with years serving as replicates.

[b] Treatments followed by the same letter do not differ at the 5% level of significance, and

[c] Treatments followed by the same number do not differ at the 1% level of significance according to Duncan's multiple range test.

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DEPARTMENT OF PLANT PATHOLOGY, NEW JERSEY AGRICULTURAL EXPERIMENT

EFFECT OF HERBICIDES ON NEMATODES IN BENTGRASS[1]

Wilbur D. Courtney, Dwight V. Peabody, Jr., and H. M. Austenson[2]

Previous work in western Washington showed that herbicides were effective for the control of the bentgrass nematode[3]. Subsequently, a number of chemicals have been shown to affect grass growth specifically. In the winter of 1960-61, 35 chemicals (each at four rates) were screened in the greenhouse for their effects on heading of bentgrass.

Seven of the most promising of these chemicals were applied in the spring of 1961 to an area of nematode-infested bentgrass in the field. All chemicals were applied as sprays in a total volume of water equivalent to 100 gallons per acre. The chemicals, rates of application, and heading responses are shown in Table 1. All data reported are the averages of three replications.

Table 1. Herbicides applied to colonial bentgrass May 19, 1961 and their effect on head density.

Chemical	Rate (pounds active/acre)	No. heads/sq. ft. August 1, 1961
None (Check)		333
Dalapon	5	0.69
Amitrole	5	244
do.	10	314
1,2-dihydropyridazine-3,6-dione (MH)	8	1.04
do.	16	0.68
Chlorazine	2	321
do.	4	203
4-chloro-2-butynl N-(3-chlorophenyl) carbamate (Garlon)	4	225
3,4-dichloropropionanilide (FW 734)	2	241
do.	4	294
Propylethyl-n-butylthiol-carbamate (R2061)	2	263
do.	4	333

Table 2. Effect of herbicides on nematode development in bentgrass.

Treatment (lb/acre)	% galls with nematodes	% nematode revival
Check	80.7	86.7
Amitrole 5	41.7	5.3
Amitrole 10	7.3	0.6
Dalapon 5	5.7	0.9
MH 8	24.0	11.0
MH 16	12.7	8.7

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Washington Agricultural Experiment Stations. Scientific Paper No. 2179, Washington Agricultural Experiment Stations, Pullman, Washington.

[2] Respectively, Nematologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture; Assistant, and Associate Agronomists, Washington State University.

[3] Apt. Walter J., H. M. Austenson, and Wilbur D. Courtney. 1960. Use of herbicides to break the life cycle of the bentgrass nematode, Anguina agrostis (Steinbuck 1799) Filipjev 1936. Plant Disease Reptr. 44: 524-526.

The only chemicals with any marked effect on heading were dalapon and maleic hydrazide (MH). Other materials which showed promise in the greenhouse screening test did not appreciably suppress heading in the field. Infection with nematode galls was uniform throughout the experiment area, averaging 62% of the heads.

Two samples of 0.25 square foot each were removed from each plot on August 1, 1961, and the heads were counted to determine the number per square foot (Table 1). From these samples, heads bearing the typical galls caused by nematode infection were examined for content and condition of nematodes. Previous experimental work[3] showed that two applications of amitrole at 5 and 10 pounds per acre permitted considerable heading but killed the nematodes within the galls. Data from 1961 tests of single applications of amitrole, dalapon, and MH compared with the untreated check are shown in Table 2. Points of special interest follow.

1. In the untreated check only 80.7% of the galls contained nematode larvae. This is 10 to 15% less than in similar material from commercial bentgrass fields examined in previous tests. Since remnants of adult females were found in many of these galls, possibly they had entered without males and were not able to reproduce.
2. Nematode larvae were found in 41.7% of the galls contained in the plots sprayed with amitrole at 5 pounds per acre while the 10-pound rate reduced this amount to 7.3%. Only 5.3% of the larvae for the 5-pound rate and 0.6% for the 10-pound rate of amitrole were revived, as compared with 86.7% for the untreated check.
3. Dalapon reduced the percentage of galls with nematodes from 80.7% in the check to 5.7%. The revival of these nematodes was only 0.9% compared with 86.7% in the check.
4. MH at 8 and 16 pounds per acre reduced percentage of galls with nematodes to 24.0% and 12.7% and reduced revival of these nematodes to 11.0% and 8.7% respectively.
5. The percentages of nematodes found in galls from the other treatments in the experiment did not materially differ from those found in the untreated checks.

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EFFECTS OF FUNGICIDAL DRENCHES ON ROOT INFESTATION BY RHIZOCTONIA SOLANI AND INCIDENCE OF TWO-SPOTTED SPIDER MITES ON STRAWBERRIES

E. M. Stoddard and P. M. Miller

Abstract

A soil drench with 500 ppm or 1000 ppm of 8-hydroxyquinoline sulfate controlled R. solani on strawberry roots and inhibited two-spotted spider mite infestations on leaves. A drench with captan or pentachloronitrobenzene gave slight control of R. solani, but the captan increased mite infestation. Combining soluble and insoluble fungicides is suggested for best control of soil-borne diseases on established plants.

Many soil-borne diseases are sporadic. Most growers will not invest in a preplant soil treatment to prevent sporadic diseases. Instead, growers want a treatment that will eliminate or stop the spread of soil-borne disease in established planting. Soil drenches with fungicides have been used successfully for this purpose (2).

In 1961 root rot caused by Rhizoctonia solani Kuehn damaged several commercial strawberry plantings in Connecticut. None of these had any fungicidal soil treatment before planting. A fungicidal drench was needed to save surviving plants to produce a crop for the next season. To select an effective treatment, soil drenches were tested in the greenhouse on potted strawberries infested with R. solani.

EXPERIMENTAL METHODS AND RESULTS

Individual, field-grown strawberry plants, variety Stelemaster, were each potted in paper cups containing 600 g of unsterilized sand when the roots were about 6 cm long. Each plant was then inoculated with 1 g of chopped strawberry roots naturally infested with R. solani.

Two days after transplanting, fungicidal drenches were applied. Each treatment was applied on five plants, 200 ml per plant. Fungicides and amounts used per liter were as follows: pentachloronitrobenzene (PCNB), 4.8 g of 75% wettable powder; captan, 4.8 g of 50% wettable powder; 1,2-dibromo-3-chloropropane (DBCP), 0.15 ml of 50% emulsifiable concentrate; and 8-hydroxyquinoline sulfate (OQS), 0.5 g and 1.0 g of technical grade.

Relative root infestation by R. solani was determined 8 weeks after treatment. To do this sections of young strawberry roots were removed from the sand, and shaken to free them of loose sand. The root samples were then dipped into clear lactophenol, drained, and mounted in clear lactophenol on a microscope slide. The number of mycelial fragments on 5 cm of roots from each plant was counted, to give five counts per treatment.

The effect of these treatments on infestation by R. solani is given in Table 1. Both concentrations of OQS greatly reduced the infestation. PCNB and captan reduced infestation but not satisfactorily. DBCP was ineffective.

Samples were also taken from roots which had grown out of the pots into the sterilized sand below, to see if the treatments could protect roots not in the treated sand. Counts on these roots showed that no treatment prevented infestation by R. solani once the roots were outside the pot.

In a second test only OQS was used. Amounts used per liter were 0, 1 g, and 2 g. Counts were made 11 weeks later instead of 8 weeks as in the first test to see if higher concentrations might give longer control. The results of this test were as follows: Water control--17.2 mycelial fragments per 5 cm; 1 g of OQS per liter--9.5; and 2 g of OQS per liter--6.2. Thus the higher concentration of OQS was still giving good suppression of R. solani infestation after 11 weeks. However 1 g of OQS per liter was much less effective at 11 weeks than it was after 8 weeks.

Also, the fungicidal drenches influenced leaf infestation by the two-spotted spider mite, Tetranychus telarius L. Seven weeks after the start of the experiment, leaves on all plants drenched with captan were very heavily infested with eggs, nymphs and adult mites. Only a few scattered mites were then present on nearby plants which were untreated or given other treatments. Three weeks later, mite infestations were very heavy on plants treated with captan, PCNB, and DBCP and untreated plants. However only a few adult mites and very few nymphs or eggs were on plants treated with OQS.

Table 1. Influence of fungicidal soil drenches on infestation of strawberry roots by Rhizoctonia solani.

Fungicide in drench	Amount per liter of water	Average number of mycelial fragments of R. solani per 5 cm of roots[a]
Untreated		12.7
OQS	0.5 g	2.3
	1.0 g	3.4
Captan, 50% WP	4.8 g	9.3
PCNB, 75% WP	4.8 g	7.5
DBCP, 50% emul. con.	0.15 ml	13.8
LSD 5%		3.7

[a] Each figure is average of five counts, one count on one sample from each of five pots per treatment.

DISCUSSION

A soil drench with OQS gave satisfactory control of R. solani infestation of roots of strawberries. It is already in widespread use in Connecticut as a drench in nursery and florist crops for control of various soil-borne diseases. It probably has little value as a preplanting treatment because it is inactivated rather quickly in soil. Its high cost and some other disadvantages may not permit use on food crops. The successful control by water-soluble OQS of soil-borne fungi on established plants does show that soluble chemicals should be much better than insoluble ones if quick control is needed because Munnecke (1) found soluble materials penetrated soil easier than insoluble ones. Insoluble fungicides should be satisfactory for preplanting treatments or where there is time for slow dispersion of the fungicide through the soil down to the roots. Combining a soluble fungicide, for quick control, and an insoluble fungicide, to give delayed control, might make an effective treatment.

The increase in infestations by the two-spotted spider mites after captan treatment and inhibition of mite infestation by OQS can be explained only by indirect actions of these materials on the mites. The two fungicides did not contact the leaves or mites themselves. We found in other tests that both fungicides or their decomposition products were taken up by plants after soil applications and with definite effects on the leaves. Either the treatments are making the plant itself more favorable or unfavorable for mites, or they are affecting biological antagonists of the mites. Fungicides applied on foliage were found by Stoddard, et al. (3) and others to affect mite incidence, as for example glyodin had been reported to depress mite populations. Stoddard, et al. (3) also found that spruce twigs sitting in a solution of nabam would absorb and translocate enough nabam to prevent mite infestation. However in the current test on strawberries, drenches with OQS and captan did affect infestation with two-spotted spider mites. Thus soil fungicides as well as fungicidal sprays may influence incidence of foliar pests.

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AN UNUSUAL LOCAL EPIDEMIC OF TOMATO GHOST SPOT

R. E. Webb

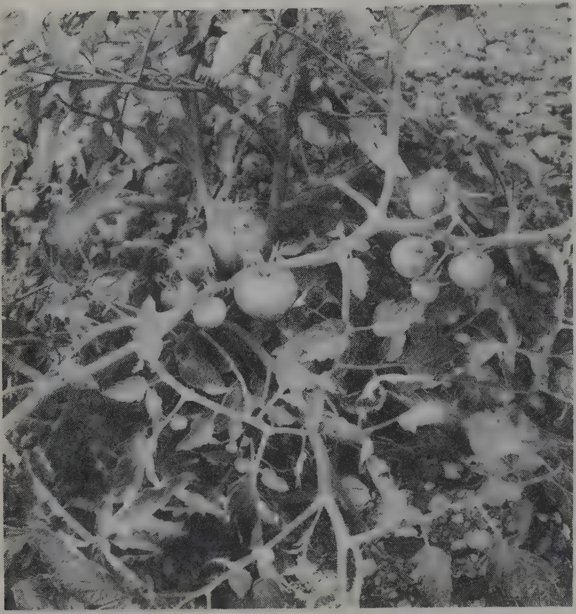

FIGURE 1. Tomato fruit on vine showing ghost spot.

Ghost spot of tomato, a disease recently found to be caused by Botrytis cinerea Pers.[1], has been occasionally noticed on tomato fruit in the field in the Atlantic and Central States and in the greenhouse. Usually the disease is not prevalent enough to be considered of economic importance. Local epidemics, however, sometimes cause concern to growers. Such an epidemic occurred in a 5-acre planting of tomato varieties and breeding lines at Plant Industry Station, Beltsville, Maryland, in early July 1961. Several fruit samples submitted from elsewhere in Maryland and from Virginia showed similar symptoms.

The disease, not apparent during a critical inspection June 29, was first observed July 3. Almost every fruit, regardless of size or position on plant, over the entire planting had a few to very many small whitish rings (Fig. 1) scattered over the entire fruit. Later formed fruit were similarly diseased. Many spots had minute pinpoint necrotic lesions at or near the centers. Tissue within the rings usually became slightly brownish-green a few days after the disease appeared, and slight necrotic flecking of the fruit flesh underneath and near the center of spots was visible to the naked eye. The whitish rings appeared to be confined to the skin layer of the fruit, and only the necrotic flecking extended into the flesh. Although wide variations in fruit spotting were noted on the approximately 150 lines and varieties bearing fruits July 3, no outstanding differences in susceptibility were apparent. Injury to foliage by Botrytis was not observed during the entire season though no fungicide was applied to the plants.

The fact that many of the fruit spots had pinpoint necrotic lesions near their centers suggested that the causal agent might be an insect. However, the plants had been dusted with an insecticide on a weekly schedule before the disease outbreak. Most of numerous isolations attempted from affected fruit yielded no organism. Alternaria sp., however, was present in approximately 2% of the transfers from the necrotic areas in the centers of the ghost spots. Inoculations of immature fruits growing in the greenhouse with the Alternaria sp. failed to induce necrosis or ghost spot.

The planting of tomatoes was adjacent to a running stream and surrounded by trees on the east and south and low-lying ridges planted to various crops on the west and north. Immediately adjacent to the tomato plot, several acres of a grain crop interplanted with vetch matured in early June. Botrytis sp. was sporulating abundantly on the lower leaves of the grain and vetch on July 3. Dead and decaying lower leaves of the grain and vetch indicated that Botrytis had been present for some time during the moist weather before the appearance of ghost spot on tomatoes.

Frequent rains had fallen and local ground fog had occurred over the area occasionally at night for approximately 2 weeks before the appearance of ghost spot. Temperature during this period averaged 55° F at night and 77° during the day.

Although frequent rains occurred throughout the summer and early fall in the Beltsville area, tomato fruit decay or foliage injury due to Botrytis was not apparent in the planting affected by ghost spot. Young, immature, as well as mature, green fruit affected by ghost spot developed to normal size, shape, and coloration characteristic of the line or variety. Ghost spot symptoms, however, were still prominent when the fruit was fully vine-ripe. Palatability of affected fruit was not adversely affected. Incidence of anthracnose fruit rot developing late in the season did not appear to be affected by the presence or absence of ghost spot.

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BOTRYOSPHAERIA TWIG BLIGHT OF ARIZONA CYPRESS[1]

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Abstract

Inoculations have demonstrated the pathogenicity of an apparently undescribed species of Botryosphaeria associated with a serious twig blight of Arizona cypress (Cupressus arizonica) in Christmas tree plantations in Georgia. The fungus differs from B. dothidea (Moug. ex Fr.) Ces. & de Not. in its larger ascospores and conidia. Differential susceptibility among host plants observed in the field and in greenhouse inoculations of seedlings indicates that resistance to Botryosphaeria twig blight may be obtained by selection.

INTRODUCTION

An undetermined species of Botryosphaeria with an imperfect stage in Dothiorella has been found associated with a destructive twig blight of Arizona cypress in Christmas tree plantations in Georgia. This twig blight disease was first observed in August 1959, in a plantation in Henry County. Although extreme variations in severity of infection occurred among individual trees, so many were rendered unsalable that no attempt was made to harvest the crop. Despite a lower level of infection in 1960 and 1961, deformation of trees severely infected in 1959 was still apparent 2 years later. This disease appears to be identical with a twig blight of Arizona cypress found in Alabama and Texas in 1941 (8). There have been no subsequent reports of this twig blight, and the causal relationship of the fungus associated with it has not been established. Although no extensive surveys have been made since rediscovery of the disease in 1959, it has been found in plantations in Brooks, Crisp, Houston, and Peach counties and was observed on Christmas trees on the market in Griffin, Georgia in 1961. In Brooks and Crisp counties it was associated with Cercospora blight (Cercospora thujina Plakidas), a disease recently reported on Arizona cypress in Arkansas and Georgia (5, 7, 9, 10).

SYMPTOMS

Severe infection with Botryosphaeria twig blight resulted in the killing and browning of stem tips over the entire surface of the tree. Only unhardened portions of the stem were affected. Consequently, the most extensive damage occurred toward the top of the tree where long, rapidly-growing leaders were exposed to infection (Fig. 1). In this region the blight extended as much as 30 cm back from the tips of some shoots. Although early infections could sometimes be distinguished as brown spots on individual leaves, the stem was rapidly invaded and death of the stem tip occurred much more quickly than in the case of Cercospora blight, which is primarily a leaf blight. Infected shoots suddenly withered, assumed a grayish cast, and then became brown. Black, spherical, erumpent pycnidia pimpled the surface of the dead tissue. Examination with a hand lens distinguishes the pycnidia of this fungus from the conidiophore tufts of Cercospora thujina. Diagnosis should be based on microscopic examination to avoid confusion with Phomopsis blight (4), although the latter disease appears to be infrequent on Arizona cypress.

THE FUNGUS

Pycnidia formed abundantly in the leaf blades and in the leaf bases clasping the stems. They were separate, globose, black bodies 154-190μ in diameter erumpent through the host cuticle. Each contained a single cavity surrounded by a thin pycnidial wall and opening by a pore through a short apical beak. Conidia were oblong to long ellipsoid, sometimes irregularly constricted or curved, hyaline, non-septate, and 24-45 x 8-13μ. Under moist conditions conidia collected in droplets at the tips of the pycnidial beaks. The droplets often had a pinkish tinge but were sometimes amber colored.

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[1] Paper No. 413, Journal Series, Georgia Experiment Station, Experiment, Georgia.

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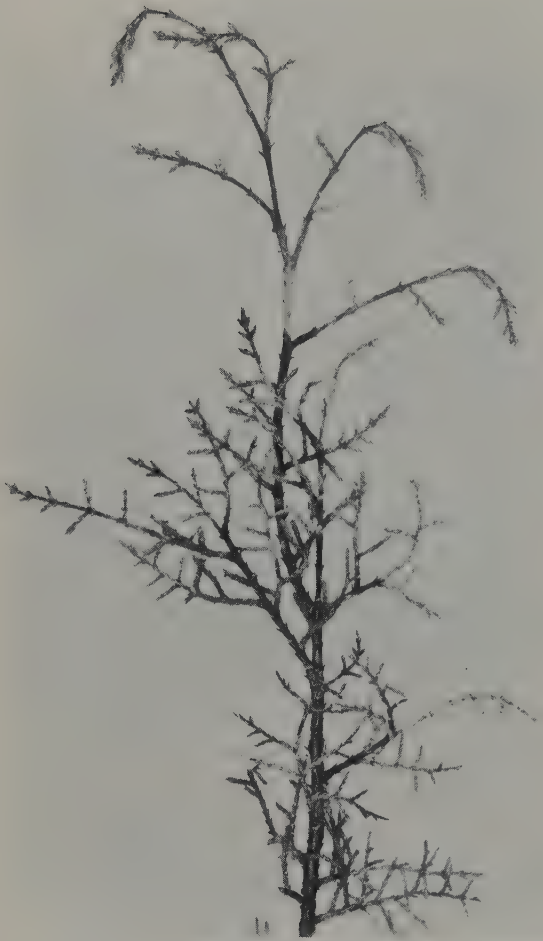

FIGURE 1. Shoot of Arizona cypress infected with Botryosphaeria twig blight.

Ascocarps developed sparsely and infrequently in the same location as the pycnidia on twigs dead for several months. A few mature ascocarps were found in the fall as well as in the spring. They were separate, slightly flattened sub-globose to flask-shaped ascostromata 210-266μ in diameter. Each contained a single locule filled with pseudoparaphyses and clavate, bitunicate asci. Asci measured 95-123 x 25-31μ. Ascospores were oblong, hyaline, non-septate, and 24-32 x 13-15μ. They were discharged forcibly through a pore in the short apical beak of the ascocarp.

Isolations were made from ascospores, conidia, twig tissue, and leaves. Isolations from 20 leaves showing only tiny brown spots or brown tips yielded the following: Botryosphaeria sp. - 10, Alternaria sp. - 6, sterile mycelia - 2, no organism - 2. Cultures of the Botryosphaeria from all sources were similar. Most favorable media for sporulation were cornmeal and oatmeal agars. Cultures were incubated under fluorescent light at 20° or 25° C. Pycnidia developed in the gray aerial mycelium or at the agar surface. They were often compound in black stromata.

Von Arx and Müller (1) reduced a great number of species of Botryosphaeria to synonymy with two species: B. quercuum (Schw.) Sacc. (Physalospora malorum Shear) characterized by large ascospores measuring 24-42 x 10-18μ and a Sphaeropsis conidial stage and B. dothidea (B. ribis Gross. & Dugg.) characterized by smaller ascospores measuring 15-26 x 6-9μ and a Dothiorella conidial stage. Shear, et al. (6) recognized this same division on the same basis. They included in P. malorum a series of forms with ascospores measuring 18-40 x 6-16μ and brown, oblong conidia measuring 17-31 x 7-15μ (Sphaeropsis), in B. ribis a series of forms with ascospores measuring 13-28 x 4-12μ and hyaline, ellipsoid conidia measuring 10-29 x 4-9μ (Dothiorella). The twig blight Botryosphaeria disrupts this correlation between ascospore size and conidial stage; the ascospores are in the size range of B. quercuum, whereas the conidial stage falls in Dothiorella, as in B. dothidea. However, the conidia as well as the ascospores are distinctively larger than those of *B. dothidea*. *B. dothidea* was found on larger dead stems and cut branches of Arizona cypress and was readily distinguished from the twig blight *Botryosphaeria*.

Pycnidia of the twig blight fungus formed on the host leaves are simple, and the imperfect stage might be placed in *Macrophoma*. As suggested by Waterman (8), it corresponds with the description (2) of *M. cupressi* Cke. & Hark. The ascocarps also are simple as in fungi that have been placed in *Physalospora* (incorrectly so, according to Von Arx and Müller (1)). Among these it agrees fairly well with the description of *P. cupressi* (B. & C.) Sacc. (3). In the type collection of *P. cupressi*, obtained from the Farlow Herbarium through the courtesy of Dr. I. Mackenzie Lamb, ascospores were 26-32 x 11-13μ and asci 98-112 x 22-28μ. This corresponds reasonably well with the twig blight *Botryosphaeria* except for the larger ascocarps (304-370μ in diameter). However, Von Arx and Müller (1), probably correctly, placed *P. cupressi* in synonymy with *B. quercuum*. Unfortunately, the conidial stage is unknown, and this alone could settle the question. Disposition of the twig blight fungus requires further study. It probably represents an undescribed species.

INOCULATIONS

Inoculations were made on potted Arizona cypress seedlings from 1 to 3 feet tall. Inoculum, consisting of a suspension of conidia and mycelium fragments from plate cultures, was sprayed onto the seedlings, which were then placed in an inoculation chamber for 48 hours. Inoculum from cultures isolated from ascospores, conidia, and host tissue gave similar results. The first symptoms of infection appeared in 2 weeks as a sudden withering and graying of the stem tips. Pycnidia appeared in the dead tissue within a few days, and the fungus was easily reisolated. Additional blight symptoms appeared over a period of several weeks, while uninoculated controls remained free from infection. The blight extended 1 to 10 cm back from the tips of the shoots. Larger branches were killed by invasion from infected laterals.

Seedlings artificially inoculated showed the same variation in susceptibility as larger trees naturally infected in the field. In a series of 23 seedlings subjected to two successive inoculations the extent of infection was as follows:

Percentage of Shoots Blighted	Number of Trees
20-40	4
10-19	4
2-9	11
Trace	4

In a single inoculation test with three Arizona cypress and three Eastern red cedar (*Juniperus virginiana*) seedlings all Arizona cypress became diseased, showing a range of infection from 10 to 75% of shoots blighted. There was no immediate evidence of infection on red cedar. After 3 months a few of the red cedar leaves were partially or completely dead and pycnidia had developed on a single leaf. At the end of 4 months one branch on one of the inoculated seedlings was blighted.

PROBLEMS OF CONTROL

Observations on the Henry County plantation in which Botryosphaeria twig blight was the only disease present indicates that this disease is potentially disastrous. Surveys are necessary to determine the extent of the disease and to estimate its importance in Christmas tree production before the necessity of control measures can be evaluated. It may be more widespread than the paucity of reports would indicate; possibly it has been confused with Cercospora blight and Phomopsis blight since the ultimate effects of all three diseases are similar.

Arizona cypress shows such extreme variations in shape, density, color, and leaf shape that selection would be desirable to develop a more uniform product. Since similar variations in susceptibility to Botryosphaeria twig blight exist, prospects seem excellent that selection for resistance can be included in the program. Further studies are necessary to determine whether resistance to Botryosphaeria twig blight may be correlated with resistance to Cercospora blight.

Spray programs developed for control of Cercospora blight (7) may prove effective against Botryosphaeria twig blight also. The economics of spraying are complicated by the fact that many trees have sufficient resistance to escape serious damage in the absence of spraying and by fluctuations in severity of infection from year to year with no basis for predicting years in which spraying might be profitable.

Even in the absence of evidence on the buildup of disease in successive plantings, it would be advisable to separate new plantations from old ones as much as possible and to practice rotation on individual sites. In this connection it would be necessary to avoid carrying the disease on nursery stock and to seek sources of inoculum on native plants. Eastern red cedar is a possible source, but survival of the fungus on this host in the field has not been demonstrated.

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ASSOCIATION OF CLADOSPORIUM WITH NEEDLE CAST OF LARCH AND SPRUCE IN THE UNITED STATES

Richard Campana and Martin Rosinski[1]

Although Cladosporium spp. are known to infect needles of Larix and Picea spp., and to cause defoliation of these trees in Europe (6), they have not been reported on needles of these or other coniferous hosts in North America. One or more species of the fungus Cladosporium were found growing from diseased, living needle tissue of European larch (Larix decidua), and both white and Norway spruce (Picea glauca and P. abies) in Maine in 1961. According to Spaulding (6), at least two species of Cladosporium cause "black molds" on foliage of a variety of trees in Europe, Asia and North America. Cladosporium epiphyllum (Pers.) Martius is reported to cause an olive-black mold on Larix decidua in Great Britain (6). C. herbarum (Lk.) Fr., although normally saprophytic, may become destructive to succulent leaves and stems, and it has been reported on species of Picea and Pinus in Russia, Pinus rigida in Germany, Thuja orientalis in China, and Pinus strobus in Yugoslavia (3, 6). C. laricis Sacc., reported to cause spotting of needles, may cause damage in both seedlings and older trees; and it has been reported on Cryptomeria japonica f. elegans, Cryptomeria viridis, Larix decidua and Pinus pinea in Italy (2, 3, 6).

It is of some interest that Spaulding (6) lists C. herbarum among semi-pathogens, which only become parasitic "under suboptimum conditions for the host tree, or under conditions especially favoring increase in the vigor of the pathogens." On the other hand, he lists both C. laricis and C. epiphyllum as wound parasites "that can attack trees only through wounds that expose inner living tissues to easy attack." A review of the literature by the present writers has failed to reveal a single record of Cladosporium spp. associated with a foliage disease of conifers in North America. In addition, we found no record of Cladosporium on foliage of Picea glauca either in America or Europe (1, 4, 5, 6, 7).

In late August of 1961 needles of planted 14-year-old European larch in the University of Maine Forest at Old Town, Maine had begun to fall permaturely[2]. Examination of several samples indicated yellowing and browning of needles in typical infection patterns, and the presence of a black-green mold was noted in the infected areas. An undetermined species of Cladosporium was positively identified by microscopic examination of conidia and conidiophores. All of the trees examined in the stand were similarly affected, with substantial needle cast occurring at least a month before normal seasonal defoliation. The foliage of native eastern larch (L. laricina) in nearby stands was unaffected. Noting the record of the fungus on European larch in Europe, we assumed that the species was probably Cladosporium laricis.

In the following week Cladosporium was identified on seedling nursery stock of white and Norway spruce in the Greenbush Nursery of the Maine Forest Service about 25 miles from the infected larch at Old Town[3]. Its occurrence on spruce was associated with yellowing and defoliation of several thousand seedlings in nursery beds. The fungus appeared to be identical with that found earlier on larch. However, Spaulding's citation (6) of the fungus on Picea in Russia indicated Cladosporium herbarum rather than C. laricis. Because species of Cladosporium are saprophytes and parasites of world wide distribution, and because we had identified the genus on foliage of numerous other woody plant species, initially we did not consider the presence of this genus on coniferous foliage to be unusual[4]. It was only when we were unable to find a previous record of Cladosporium spp. on the foliage of any species in the family Pinaceae in North America that we recognized that this represented an unrecorded association.

Examination of the affected spruce seedlings showed that only needles of the current season were infected, that infection was confined to foliage on the lower two-thirds of the stem growth for 1961, and that needles so affected subsequently became at least partially necrotic prior to premature defoliation. The needles in the terminal third of the current season's growth appeared to be unaffected. The limitation of infection to lower needles may be attributed either to a wet period, when only lower needles close to the source of inoculum on the ground had emerged from the stem, or to extended periods of moisture retention through shading and decreased desiccation from wind. The period of most stem growth and needle emergence of conifers in Maine, extending from June 1 through July 15 in 1961, was unusually wet, cool and humid. Optimum climatic conditions for mass development of fungus spores prevailed at the same time that emerging, coniferous needle tissue was exposed to fungus infection.

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[1] Professor of Botany and Associate Professor of Botany, Department of Botany and Plant Pathology, University of Maine, Orono, Maine, respectively.

[2] The diseased condition of larch was detected and reported to the writers, and the original specimens of the fungus on larch were collected by Professor Henry A. Plummer of the School of Forestry of the University of Maine.

[3] The diseased condition of spruce was reported to the writers, and the original specimens of spruce were collected by Mr. Fred Atchison, Supervisor of the Greenbush Nursery of the Maine Forest Service.

[4] Unpublished data in pending manuscript. Cladosporium had been identified previously in Maine only on angiosperms.

Since the record of Cladosporium spp. as a cause of disease in needles of conifers in Europe indicates that the fungus in primarily saprophytic, it appeared that damage to larch saplings and spruce seedlings now affected may be temporary unless optimum climatic conditions for the fungus occur again during the growing season of 1962. On potted spruce seedlings defoliation of affected needles continued for several weeks until all yellow needles had fallen. The pattern was consistent, involving loss of the lower one-half to one-third of the needles of the current year. Further observations are planned to identify one or more species of Cladosporium, to establish, confirm or reject Cladosporium spp. as primary agents of disease, and to follow progress of foliage now affected.

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MAINE AGRICULTURAL EXPERIMENT STATION, ORONO

INCOMPATIBILITY OF PRICE AND COOK NUCELLAR EUREKA LEMON TREES ON NO. 1452 CITRUMELO ROOTSTOCK

J. D. Kirkpatrick, W. P. Bitters[1], and F. J. Foote[2]

Summary

Propagations of Price and Cook Nucellar Eureka lemons (Citrus limon) on No. 1452 citrumelo (Poncirus trifoliata x Citrus paradisi) have developed a bud-union crease, declined, and collapsed. The bud-union disorder is tentatively considered as a physiological incompatibility.

INTRODUCTION

In lemon rootstock experiments planted in 1959, young trees of Price and Cook Nucellar Eureka lemons (Citrus limon) propagated on No. 1452 citrumelo (a hybrid of Poncirus trifoliata x Citrus paradisi) developed a bud-union crease, declined, and collapsed. Not all the affected trees died. Many were severely declined or collapsed by the end of the second season in the orchard, 3 years from budding. The symptoms of the disorder were apparent in the 1-year-old budling and became very pronounced by the end of the first season in the orchard, 2 years from budding.

SYMPTOMATOLOGY

The initial external indication of the disorder occurred in the first year. It was a collar-like enlargement of the scion immediately above the bud union. When a small bark strip was removed from across the bud union, gum deposits in both the xylem and inner bark were observed at the juncture of scion and rootstock. Concurrently, a shallow suture was formed at the union precisely in the zone of the gum deposits. There was no immediate discernible effect on scion top growth and vigor.

By the second year the suture deepened and widened into a distinct crease which girdled the tree. During this period, the tree condition declined. As the scion lost vigor, typical girdle patterns appeared in the leaves followed by a general loss in chlorophyll throughout the leaf.

The bud-union crease was strongly expressed by the third year (Fig. 1). While the diameter of the scion above the crease enlarged at a rate greater than that of the rootstock, there was no increase in diameter at the bud union. The trees were in a severe stage of decline and collapse.

DISCUSSION

The initial stages in the development of the bud-union crease and the decline and collapse of the tree were similar to those described for other Eureka lemon trees propagated on some of the citranges, hybrids of Poncirus trifoliata x Citrus sinensis, (1, 2). Symptom development, however, proceeded at a greater rate and the bud-union crease was more pronounced for Price and Cook Nucellar Eureka lemons propagated on No. 1452 citrumelo than it was for Cook Nucellar Eureka lemon propagated on Troyer citrange. The comparison was based on trees propagated from the same budwood source and grown under nearly identical conditions.

All of the many Eureka lemon strains tested on Troyer, Savage, Morton, and Cunningham citranges have failed. They developed similar bud-union creases and the trees declined and many collapsed. The bud-union crease of Price and Cook Nucellar Eureka lemons on No. 1452 citrumelo, although more pronounced, appears to be of the same general character as that of the bud-union crease found with the Eureka lemon strains tested on Troyer, Savage, Morton, and Cunningham citranges. It is therefore probable that other Eureka lemons propagated to No. 1452 citrumelo will develop similar bud-union difficulties.

This is a report of another instance in which a hybrid of P. trifoliata failed as a suitable rootstock for the Eureka lemon variety. Not all hybrids of P. trifoliata, however, are

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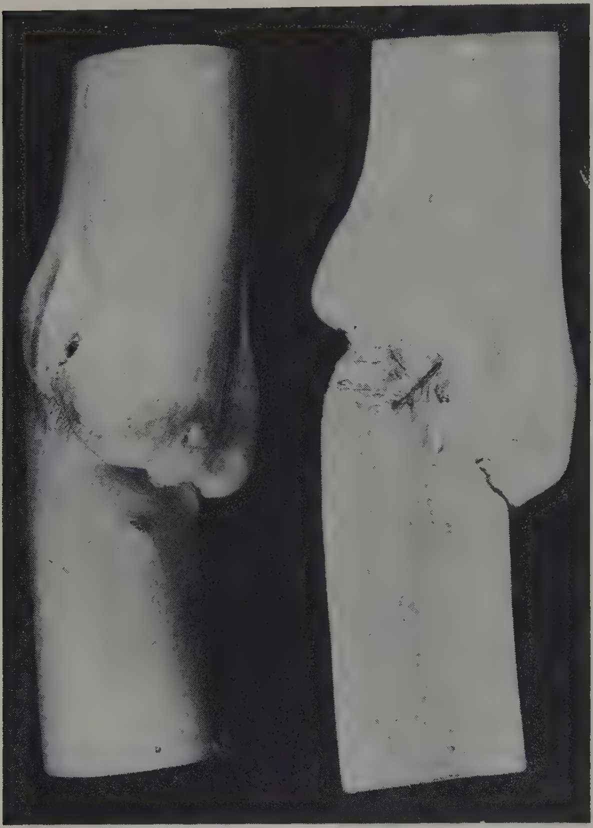

FIGURE 1. Radial section of a typical bud union of 2-year-old Cook Nucellar Eureka lemon trees on No. 1452 citrumelo rootstock that showed decline and collapse.

incompatible with all lemons of the Eureka variety, nor are all lemon varieties incompatible with No. 1452 citrumelo and the citranges tested. For example, No. 1449 citremon (a hybrid of P. trifoliata x C. limon) has made a satisfactory rootstock for 12 years budded to the Frost Nucellar Eureka lemon, and it also appears to be equally good with healthy old lines and seedling lines of the Lisbon and Villafranca lemon varieties. Troyer, Carrizo, Morton, and Savage citranges, while incompatible with all strains of the Eureka lemon variety tested, appear to be compatible and are making satisfactory rootstocks for healthy old lines and seedling lines of Lisbon and Villafranca varieties. This emphasizes that each promising rootstock selection must be tested with all varieties of a scion species. Predictions of stionic reaction based on the results with one of several varieties of a species or on the parentage of the rootstock are premature.

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A CHLOROTIC DECLINE OF PONDEROSA PINE IN SOUTHERN CALIFORNIA[1]

J. R. Parmeter, Jr.[2], R. V. Bega[3], and T. Neff[4]

Summary

A chlorotic decline, first noted in the early 1950's, has affected ponderosa pine over several thousand acres in the San Bernardino Range. The decline is characterized by progressive reduction in terminal and diameter growth, loss of all but the current-season's needles, reduction in number and size of needles, yellow mottling of the needles, deterioration of the fibrous root system, and eventual death of trees. Examination of and isolation from roots, stems, and needles failed to disclose the consistent presence of pathogenic organisms. Drought, air pollution, or a combination of the two are suggested as possible causes.

The Arrowhead-Crestline area of the San Bernardino National Forest supports some of the finest stands of ponderosa pine (Pinus ponderosa) in southern California. These stands provide, in addition to traditional timber values, vital watershed for the Los Angeles area and recreational facilities for more than 4 million visitors each year.

In the 1950's scattered trees within these stands began to show symptoms of decline. The presence of the black pine-leaf scale, Nuculaspis californica Coleman, prompted a survey to determine if the decline was associated with this insect. In a roadside survey, Stevens and Hall (8) examined trees on 184 plots in the affected area. They found no correlation between scale infestation and decline. Since the cause of the disease remained unknown, the name "X disease" was adopted by foresters. This disease has continued to intensify, and damage to pine stands has increased rapidly during the past 5 years. Several thousand acres are presently affected.

SYMPTOMS

The symptoms of chlorotic decline involve changes in both growth characteristics and needle color. The first recognizable change in growth is the loss of all but the current-season's needles, as contrasted with the normal 3 to 5 years' needle retention. This loss of needles gives the branches a tufted appearance, the needles of the tufts often twisting upward. As the decline continues, both the length and the number of needles per growth segment are progressively reduced until only a few dwarf needles are found at the branch tips. Progressive reduction in both terminal and diameter growth accompanies this decline (Figs. 1 and 2). In the final stages, the branches die, usually from the bottom of the crown upward. The leader and uppermost branches are the last to die. The average duration of decline is not known, but growth measurements and field observations indicate that at least 3 to 5 years or more of decline normally precedes death of a tree. Figure 3 shows a tree in the final stages of decline.

Marked changes in needle color also accompany decline. The needles on diseased trees develop a characteristic yellow mottling that, in advanced stages, imparts a bright yellow cast to the entire crown. Such trees stand out in obvious contrast to normal green trees. A close examination of needles shows that the yellowing is a result of numerous chlorotic blotches or bands that develop on all needle surfaces. As the chlorotic blotches become more numerous, the needles become progressively more yellow until only faint patches of green remain.

The degree of chlorotic mottling varies with the season and with the age of the needles. In early spring, only mottled current-year's needles are present on diseased trees. The new needles, produced during early summer, are uniformly green and remain so until late August or September, when faint mottling begins to appear. During the fall and winter, the old mottled needles are shed and mottling increases on the new needles. By the following spring, diseased trees again show only mottled current-year's needles. Thus, during part of the summer

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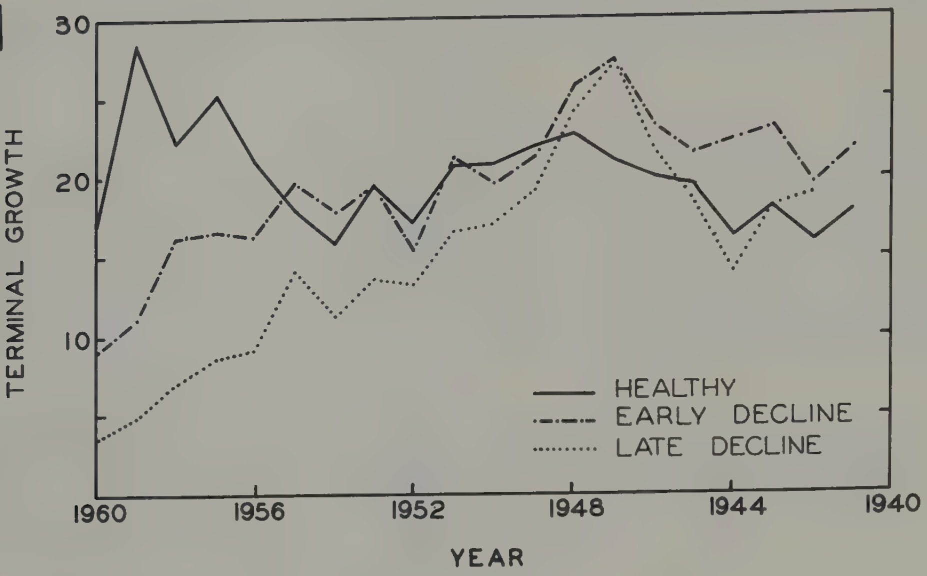

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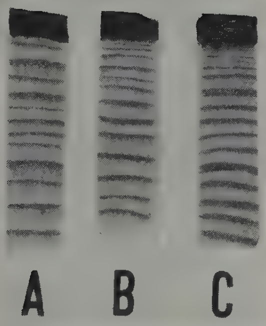

FIGURE 1. Average annual terminal growth (in centimeters) for 11 healthy trees, 8 trees in the early stage of decline, and 5 trees in the late stage of decline.

FIGURE 2. Stem sections from a healthy tree (A), a tree in the early stage of decline (B), and a tree in the late stage of decline (C).

FIGURE 3. A tree in the late stage of decline, showing thin crown, dwarfed needles, and absence of all but the current season's needles.

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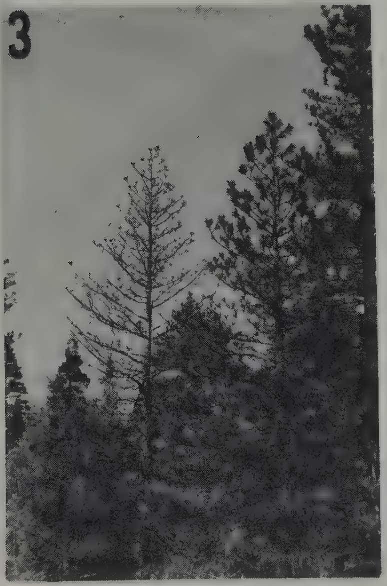

and fall, diseased trees show yellow year-old needles and green current-year's needles. During the remainder of the year, only mottled current-year's needles are present. The intensity of mottling varies on trees in similar states of decline, some showing more pronounced yellowing than others. Yellow mottling also occurs on the older needles of some vigorous trees with 3 to 5 years' needle retention. Whether this represents a very early stage of decline is not known.

In addition to chlorotic mottle, many ponderosa pines in the area also show a needle dieback. This dieback, involving the reddening and death of the tips of scattered needles, follows much the same pattern of development as that described by Linzon (6) and Berry (1) for emergence tipburn of Pinus strobus. Needle dieback usually affects only scattered needles and crown symptoms are slight. Occasional trees show severe dieback, however, and the crowns of such trees are strikingly red. Needle dieback is common on both vigorous trees and trees showing chlorotic decline. Many trees in various states of decline show little or no dieback, however. Repeated examinations and isolation attempts have failed to disclose any association of pathogens with needle dieback.

OCCURRENCE AND DISTRIBUTION OF CHLOROTIC DECLINE

Chlorotic decline has been observed only on ponderosa pine and only in the San Bernardino Range. Other tree species common in the area -- white fir (*Abies concolor*), incense cedar (*Libocedrus decurrens*), sugar pine (*Pinus lambertiana*), Jeffrey pine (*Pinus jeffreyi*), bigcone Douglas fir (*Pseudotsuga macrocarpa*), and Coulter pine (*Pinus coulteri*) -- have shown no symptoms of decline. Ponderosa pines of all ages are affected. Diseased trees occur randomly in residential areas, undisturbed timber stands, and stands that have been cut-over one or more times. The disease also occurs randomly and with equal frequency on ridge tops, in valley bottoms, and on slopes of all exposures. Thus the occurrence of the disease within the Arrowhead-Crestline area does not appear to be correlated with topographical or site factors, with age, or with stand conditions. Dead and dying trees are often found with branches intermingled with those of adjacent healthy trees, and diseased trees show no tendency to occur in groups.

PROGRESS OF CHLOROTIC DECLINE IN TIMBER STANDS

In September 1957, a 2-acre plot near Enchanted Valley was established in an all-aged, site II, timber stand to record the progress of the disease. This stand had been thinned in 1953, and at that time 95% of the trees were classified as Keen classes A and B, indicating that the stand was vigorous and in good condition in 1953. In 1957, 145 trees were tagged, measured for dbh and height, and given a disease rating. Three classes of trees, healthy, early decline, and late decline, were recognized on the basis of needle length and color, needle retention, and general condition of the crown. The trees were inspected annually to determine changes in disease condition. Table 1 shows the changes that occurred between 1957 and 1960. During this period the number of trees in the late decline class (including trees that had died) rose from about one-third to nearly two-thirds of the plot trees. Mortality was 10%. These data indicate the rapidity with which the disease affects timber stands and the potential of the disease to damage stands. Observations in the Arrowhead-Crestline area indicate that the incidence of the disease in other pine stands is similar to that in the plot.

Table 1. Changes in the incidence of chlorotic decline of ponderosa pine on a permanent sample plot during the interval 1957 to 1960.

Diameter class (inches)	Condition of trees							
	symptomless		early decline		late decline		dead	
	1957	1960	1957	1960	1957	1960	1957	1960
4-8	9	5	14	7	10	16	0	5
9-13	18	6	17	14	25	34	0	6
14-18	9	4	9	10	10	14	0	0
19-23	3	0	8	2	6	12	0	3
24+	3	2	2	2	2	3	0	0
Totals	42	17	50	35	53	79	0	14
% of all trees	29	12	34	24	37	54	0	10

INVESTIGATION OF POSSIBLE CAUSES OF CHLOROTIC DECLINE

Needle Studies: Needles on diseased and healthy trees and on the ground beneath trees were examined throughout the year. No evidence was obtained to suggest that needle fungi were associated with the mottling. Needles, both surfaced-sterilized and untreated, were plated on water agar and observed for fungal growth. Again no fungi were found associated with the diseased tissues. As a final check, diseased needles were fixed in FAA, imbedded in paraffin, and microtomed sections stained with safranin and fast green or with Fleming's triple stain (safranin, crystal violet, and orange G.) were examined for the presence of mycelium. No evidence of fungal invasion was found.

Root Studies: The symptoms of chlorotic decline of ponderosa pine are similar in many respects to the littleleaf disease of shortleaf and loblolly pines (3), pole-blight of western white pine (5), and chlorotic dwarf of eastern white pine (9). Since deterioration of the fibrous root system is associated with these diseases, root excavations were undertaken to determine whether a similar deterioration was associated with chlorotic decline. Portions of the root systems of 12 diseased ponderosa pines were uncovered by shovelling. Extensive deterioration of the fibrous root system was found. Most rootlets less than 2 mm in diameter were dead and rotted. Larger roots were sound. The root systems of eight nearby healthy trees, similarly examined, showed no extensive rootlet mortality. In addition, mycorrhizae were prevalent on the roots of healthy trees but were scarce on the roots of diseased trees. Subsequent examinations of the roots of other healthy and diseased trees showed that rootlet mortality was consistently associated with chlorotic decline. Isolation studies were, therefore, initiated to determine if any root pathogens were present in or near dead and dying rootlets.

Rootlets from ten diseased trees and five healthy trees were surface sterilized for 10 minutes in a 1:10 dilution of Clorox, rinsed in sterile, distilled water, and plated on 2% water agar. In a duplicate series, rootlets were washed in sterile distilled water and plated without surface sterilization. As fungus growth appeared, mycelial transfers were made to tubes of PDA. A total of 716 isolates was obtained.

No qualitative difference in the microflora of the rootlets of healthy and diseased trees was found. Representatives of common genera of soil fungi, Mortierella, Mucor, Penicillium, Cylindrocarpon, and Trichoderma were prevalent on both healthy and diseased roots. Species of Phoma, Pyrenochaeta, and Pythium were obtained less frequently. The most prevalent fungus was an unidentified grey, sterile fungus. None of the fungi isolated was judged by occurrence or by known pathogenicity to account for the root damage.

The similarity of chlorotic decline to littleleaf prompted special attempts to determine whether Phytophthora cinnamomi Rands was present on roots or in the soil. Two series of studies were made using both avocado (11) and apple (2) as substrates. P. cinnamomi was not obtained by either technique in either of the two series of isolations.

Climate: Weather records (from Climatological Data, California) were examined to determine if any unusual climatic conditions might be associated with the decline. The Squirrel Inn #2 station, located within the decline area, provided the longest unbroken record available. Mean annual precipitation at this station is 41.24 inches. Table 2 shows, by 5-year intervals, the deviations from this mean over the past 50 years. The past 15 years show a net deficit of 90.39 inches. In addition, 1947 and 1953 were the driest years on record. Thus the area has received subnormal precipitation for a prolonged period. Figure 1, showing terminal growth of healthy and diseased trees, indicates a marked decline in tree growth corresponding to the recent decline in precipitation. Healthy trees began to regain vigor in 1955, following substantial precipitation in 1952 and 1954. Diseased trees continued to decline, however. These observations suggest that drought may be involved in the decline. The pattern of distribution and the type of symptoms are not typical of drought injury, however, and none of the associated tree species have shown evidence of similar injury.

Table 2. Deviations from the mean annual precipitation in the Lake Arrowhead-Crestline area. Deviations are totaled by 5-year intervals.

Years	Net deviation in inches
1911-15	+18.81
1916-20	+ 7.73
1921-25	+22.57
1926-30	-12.12
1931-35	-18.41
1936-40	+58.87
1941-45	+41.94
1946-50	-40.79
1951-55	-16.06
1956-60	-33.53

Air Pollution: The front of the San Bernardino Range forms the northeast rim of the Los Angeles basin. The inversion layer that develops frequently over this basin causes the accumulation of polluted air, usually referred to as smog, in the basin. Coffin (4) has shown that during daylight hours, convection from the heated mountain fronts disrupts the inversion layer at the edge of the basin and carries the polluted air to the crest of the mountains. Repeated observations from the Twin Peak's lookout showed frequent daytime movement of polluted air into the Arrowhead-Crestline area in the manner described by Coffin, and smog sufficiently dense to obscure vision and cause eye irritation is often encountered in diseased stands. Since smog is damaging to many plants (7), its possible role in chlorotic decline is being investigated.

DISCUSSION

The cause of chlorotic decline of ponderosa pine is unknown. The random pattern of its occurrence and the apparent absence of pathogenic organisms suggest that the cause is physiological. Trees in the Arrowhead-Crestline area have been subjected both to prolonged drought and to air pollution, either of which could cause physiological disturbances. Pole-blight, a decline of western white pine, is thought to result from moisture stress (5). Recently ozone, a constituent of smog, has been suggested as a cause of emergence tipburn (1), a symptom considered by Toole (10) to precede the chlorotic dwarf of eastern white pine. It is of interest in this connection that a needle dieback similar to emergence tipburn also occurs in the area where chlorotic decline is prevalent. Its relationship to chlorotic decline is unknown, however.

The occurrence of both drought conditions and air pollution in the Arrowhead-Crestline area, coupled with the likely association of these conditions with similar declines in other tree species, suggest that one or both of these conditions may be involved in chlorotic decline of ponderosa pine.

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VARIATION IN VIRULENCE OF VALSA LEUCOSTOMA[1, 2]

David S. Wysong and Lester E. Dickens[3]

Abstract

Eight isolates of Valsa leucostoma (Pers.) Fr. showed wide differences in virulence on five varieties of peach trees. The total area of necrotic tissue surrounding each site of inoculation served as a measure of virulence. Variation in virulence among the isolates explains, in part, why some orchards are more seriously affected by gummosis than others.

INTRODUCTION

Peach tree gummosis, caused by V. leucostoma, is one of the most destructive diseases affecting Prunus persica. The varying degrees of destructiveness of this disease and its erratic occurrence in peach orchards of western Colorado have given the impetus for this investigation of whether variation in virulence of the fungus exists in this area. Recently, Helton and Konicek[4], in Idaho, found several variants of V. leucostoma and V. cincta that showed differences in virulence on peach, prune, and plum trees.

MATERIALS AND METHODS

Eight isolates of V. leucostoma were obtained from peach trees in orchards near Grand Junction, Colorado in May 1960. Isolations were made from bark tissue removed from the outer margins of active cankers. Pure subcultures on PDA were used in later experiments.

Five varieties of peach trees were inoculated with each of the eight pure culture inocula. These varieties were Standard Elberta, J. H. Hale, Fair Haven, Red Haven, and Rochester, all grown in experimental plots of the Wagner orchard west of Grand Junction. The location of the experiment was selected because of its isolation from other possible sources of wild inoculum.

Trees were inoculated by cutting a flap of bark with a modified Jones patch budder (Fig. 1) and inserting a 5-mm disc from an actively growing culture of Valsa beneath the wound flap.

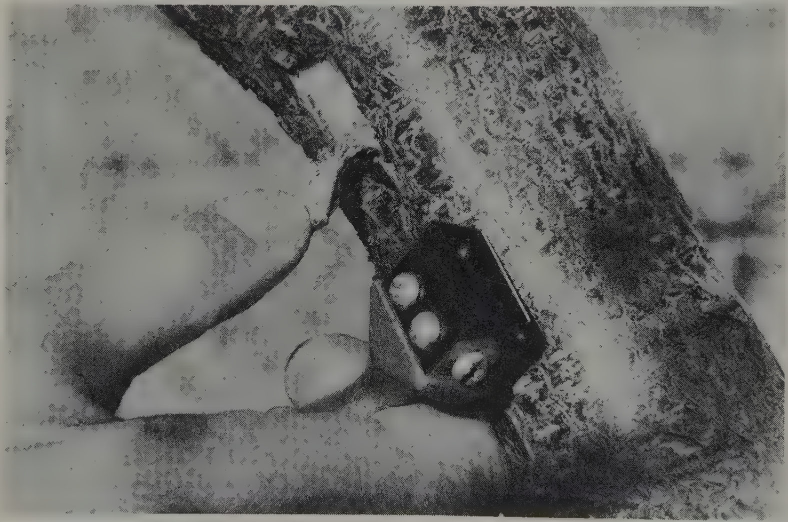

FIGURE 1. Wound made with Jones patch budder with one cutting edge removed, illustrating method of artificial wounding.

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[2] Based on a portion of a M. S. thesis submitted by the senior author to the Graduate School, Colorado State University.

[3] Graduate Research Assistant, and Assistant Professor and Assistant Plant Pathologist, respectively, Department of Botany and Plant Pathology, Colorado State University.

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FIGURE 2. Healthy tissue is separated from necrotic tissue which is exhibited by discolored zones surrounding three sites of inoculation.

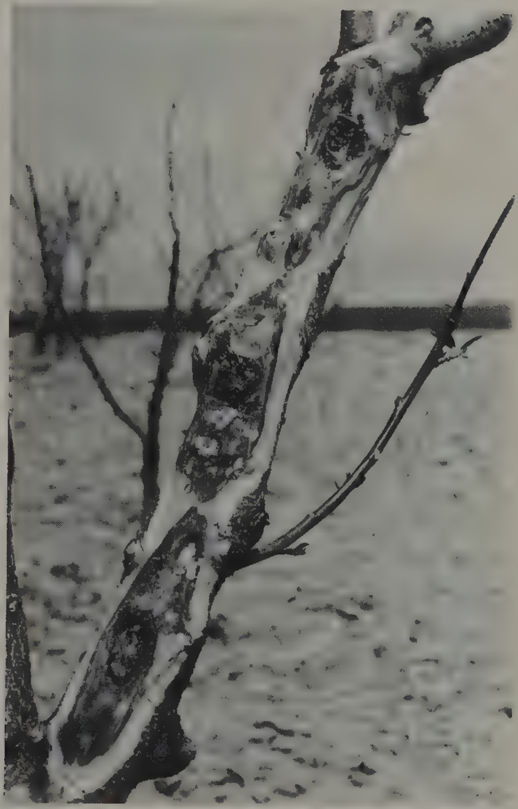

FIGURE 3. Comparison of virulence expressed as the average area in square centimeters of necrotic tissue per isolate of V. leucostoma on five varieties of peach trees.

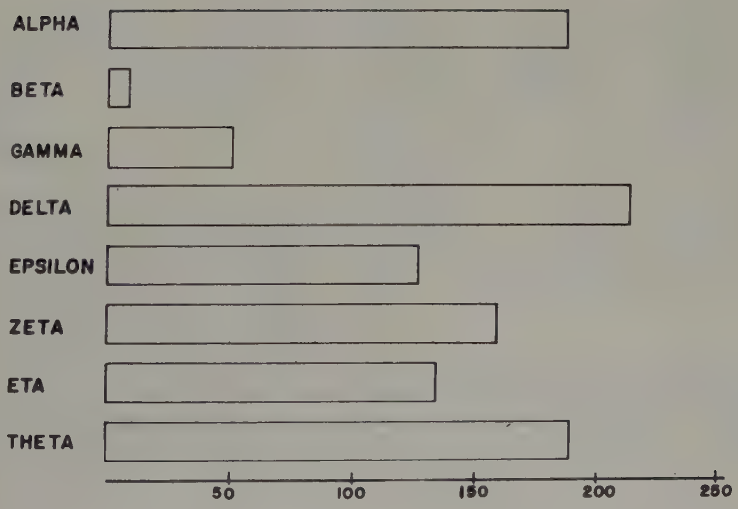

Table 1. Variation in virulence among eight isolates of V. leucostoma on five varieties of peach trees.

	Varieties						
Isolates	Standard Elberta	J. H. Hale	Fair Haven	Red Haven	Rochester	Total	Average
Alpha	107.95[a]	229.00	175.25	150.55	266.00	928.75	185.75
Beta	8.10	3.95	10.15	9.90	9.20	41.30	8.26
Gamma	10.55	107.90	10.30	42.75	81.50	253.00	50.60
Delta	86.80	213.15	212.75	156.45	390.40	1059.55	211.91
Epsilon	204.05	87.40	123.95	143.50	72.20	631.10	126.22
Zeta	89.85	-----[b]	173.65	161.85	208.35	633.70	158.43
Eta	217.20	95.30	177.15	149.60	26.95	666.20	133.24
Theta	241.50	145.85	127.05	211.95	212.10	938.45	187.69
Total	966.00	882.55	1010.25	1026.55	1266.70	5152.05	
Average	120.75	126.08	126.28	128.32	158.34		

[a] Area in square centimeters of discolored tissue measured by means of a Keuffel and Esser planimeter. Average of four replications.

[b] Premature death of limb prevented accurate reading.

Each cut thus exposed bark, cambial tissues, and an area of xylem. This technique brought the inoculum into direct contact with active cambium and xylem. The bark flap was pressed into place over the inoculum and fastened. Control trees received sterile agar discs. The inoculations were made on August 5, 1960 and measurements were recorded on April 19, 1961.

Variation in virulence was evaluated by measuring the area of tissue discoloration produced beneath the bark at each point of inoculation. The bark was removed from the wound sites to expose the necrotic areas, which were dark brown and separated from healthy tissue by sharp lines of demarcation (Fig. 2). The discolored zones were outlined on tracing paper and the total area of necrotic tissues of each inoculation site was measured with a planimeter.

EXPERIMENTAL RESULTS

Variation in virulence among eight isolates of Valsa causing gummosis on the five varieties of peach trees are shown in Table 1. The areas of diseased tissues expressed as a result of fungus activity vary widely among varieties when infected by any individual isolate of V. leucostoma. An average amount of diseased tissue caused by all isolates on individual varieties were similar with the exception of that on Rochester. Apparently Rochester is appreciably more susceptible than the other varieties tested.

A comparison of virulence among the eight isolates of Valsa on all varieties tested is shown in Figure 3. The area enclosed by each bar represents area in square centimeters of necrotic tissues. Extent of necrotic tissue was relatively small where trees were inoculated with Beta and Gamma isolates, indicating avirulence of the pathogen. There is a greater degree of virulence exhibited by Alpha, Delta and Theta isolates, however. All control trees healed properly, exhibiting no infected tissue.

DISCUSSION

Differences in virulence of eight isolates of V. leucostoma upon five varieties of peach trees were evident. The fact that various isolates differ in virulence may explain, in part, why orchards in some localities are more seriously affected by gummosis than are orchards in other localities. A grower whose trees are subject to attack by Beta or Gamma isolates may have less difficulty with gummosis than would another orchardist whose trees were infected with Alpha, Delta, and/or Theta isolates. Varying degrees of susceptibility expressed by the host is a further consideration.

The method described for measuring the discolored zones of diseased tissue on peach trees provides an accurate and efficient means for making comparisons.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, COLORADO STATE UNIVERSITY,
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THE COMPARATIVE RESISTANCE OF SEVERAL INBRED CORN LINES TO BACTERIAL WILT AND LEAF BLIGHT[1]

J. L. Peterson and J. C. Anderson[2]

Summary

Bacterial leaf blight has been the major leaf blight of field corn in New Jersey for the past 2 years (1960-1961). The effect of planting time on the incidence of wilt and the comparative resistance of several field corn inbreds to this disease were tested in 1961. Fifty-nine inbreds were planted at four different planting times at 5-day intervals in randomized and replicated plots. The incidence of the disease was quite uniform and severe throughout the test area. Planting time had no effect on disease incidence. The inbreds varied widely in expression of disease symptoms. Several lines were quite resistant to the wilt and leaf blight phases of this disease.

INTRODUCTION

During the 1960 and 1961 growing seasons bacterial leaf blight, caused by the bacterium *Xanthomonas stewartii* (E. F. Smith) Dows., was the major leaf blight observed on field corn in New Jersey. This disease was also severe in untreated sweet corn fields during this period.

The symptoms of this disease vary with the type of corn. Sweet corn develops a characteristic vascular wilt, stunt, and usually fails to develop ears when infected at an early stage of growth. Most dent corn varieties show only a leaf blight phase which develops generally after tasseling. Inbred corn lines vary in their expression of symptoms, with the more susceptible inbred lines showing the same symptoms as found in sweet corn.

The pathogen is spread principally by the corn flea beetle. The two winters preceding the 1961 growing season were either mild or had sufficient snow cover to allow flea beetles to overwinter. Consequently, conditions were right for a high incidence of bacterial wilt and blight in corn during the following growing seasons.

This paper presents data on the comparative resistance of several inbred corn lines to the wilt and leaf blight phases of this bacterial disease.

PROCEDURE

In 1961, a collection of 59 field corn inbreds used for breeding purposes were planted in randomized and replicated plots. In most instances, corn was planted at four different planting times with 5-day intervals between each planting date. The first planting was on May 21. Each replication contained 10 plants. Incidence of the disease depended on natural infection.

Readings on the incidence of wilt were made during the first week in July. Leaf blight readings were taken at two different times, the last week in August and 10 days later during the first week of September. Leaf blight incidence was based on a numerical rating from 0 to 10, 0 representing plants free from leaf blight and 10 representing plants with all leaves completely blighted. The wilt percentage was based on the number of plants per replication showing wilt symptoms.

RESULTS AND DISCUSSION

Incidence of the disease was quite uniform and severe throughout the plot. Date of planting had no apparent effect on the amount of wilt or blight symptoms in a given inbred. Since there was no difference in disease incidence in relation to planting time, each inbred average included all dates of planting. The results of this test are given in Table 1.

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers - the State University, New Brunswick, New Jersey.

[2] Assistant Professor of Plant Pathology and Professor of Farm Crops, respectively.

Table 1. Percentage of plants with wilt and leaf blight ratings of several corn inbreds[a].

	%	Leaf blight rating[c]			%	Leaf blight rating[c]	
Inbred	bacterial wilt[b]	1	2	Inbred	bacterial wilt[b]	1	2
A619	27.0	4.6	9.0	Pa83	0	2.6	3.9
B14	54.0	6.7	8.7	Pa91	0	2.5	3.0
B37	4.8	2.9	5.1	Pa415	1.0	2.3	4.5
B38	9.3	4.8	6.6	Pa419	0	1.7	4.0
B46	1.0	5.1	9.1	Pa881P	0	2.0	6.3
C103	0	3.0	6.0	Pa887P	0	1.3	3.1
C114	.5	2.3	4.9	Pa890P	3.0	2.1	---
C123	4.0	2.4	8.0	Va13a	0	2.5	6.6
H49	3.0	2.8	6.2	Va14a	4.0	2.6	7.0
H55	0	1.5	4.0	Va15a	0	2.2	6.4
H59	0	3.5	6.3	Va17	0	2.6	4.5
H60	0	1.6	5.3	Va18	4.0	4.3	6.3
J22	0	4.0	8.2	Va25	17.5	3.8	8.0
J47	0.3	3.0	7.0	Va27	0	2.0	4.9
J47ms	0	2.5	5.3	Va31	1.0	4.8	8.3
J48	1.0	2.9	5.2	Va32	0	4.9	7.9
J554	5.3	5.1	7.9	Wf9	0	3.9	8.2
MS109	0	3.3	8.4	W10	3.0	4.9	8.0
N15	0	5.1	9.0	W22	0	4.7	8.0
N38	5.0	6.3	8.0	W32	1.0	4.3	7.5
Oh07	0	3.0	5.5	W64A	3.7	4.7	8.0
Oh26	0	2.5	8.3	W153R	0	5.5	10.0
Oh43	11.0	3.3	7.0	7-94-5	7.7	3.2	7.5
Oh43E	13.0	3.0	6.3	8-359b	2.5	3.2	5.4
Oh43Tfa	15.0	4.2	7.0	38-11	0	4.8	7.5
Oh51ARf	0	2.8	7.5	91-5-1	19.0	3.3	6.6
Oh51ATms	0	3.5	9.7	91-6	2.5	3.6	5.2
Pa32	2.8	6.1	10.0	482-4A	0	3.8	7.6
Pa70	0	2.0	4.0	504-1	1.0	4.4	6.6
				504-2	21.0	4.5	7.0

[a] Inbred averages include all dates of planting.

[b] Reading taken during first week of July.

[c] Readings taken during last week of August and first week of September.

Both wilt and leaf blight symptoms vary with the inbred corn line. The inbreds included in this test showed wide differences in their susceptibility to the wilt and leaf blight phases. Lines that had a high incidence of wilt generally gave a high leaf blight reading. However, several lines which showed no wilt had a high incidence of leaf blight. Lines that had a final leaf blight reading below 4.0 were considered quite resistant. Several lines, particularly in the Pennsylvania group, were quite resistant to both phases of the disease.

Several other inbred lines were included in the planting for breeding purposes. Disease ratings were taken on these lines, but since there were only one or two replicates, these data are not included in this paper, although they are available on request.

DEPARTMENT OF PLANT PATHOLOGY, RUTGERS - THE STATE UNIVERSITY, NEW BRUNSWICK, NEW JERSEY

ZONATE RINGSPOT, A NEW VIRUS DISEASE OF BLEEDING-HEART, CLERODENDRUM THOMSONIAE

Harry C. Burnett and Charles O. Youtsey[1]

Bleeding-heart, Clerodendrum thomsoniae, is a rather widely grown ornamental plant in the southern half of Florida. Being a vine, it is primarily trained to grow on a trellis, but it may be found growing uninhibited as part of the landscaping.

In October 1961, Mr. Charles O. Youtsey, while inspecting a nursery at Sanford, Florida, noted a ringspot condition on four small C. thomsoniae plants. This material was sent to the Plant Pathology Laboratory at Winter Haven, Florida for identification of the cause of this condition. In November 1961, he found several more plants with identical symptoms from a nursery at Lake Monroe, Florida; these were also forwarded to the Plant Pathology Laboratory.

At the outset, the symptoms expressed were suggestive of those caused by virus infection in other plants; however, no such disease in the genus Clerodendrum seems to have been reported.

Leaves showing this condition were grafted on disease-free plants of C. thomsoniae. Seventeen days later the first vein-clearing symptoms showed up in the new growth.

The owners of both nurseries involved were contacted in an effort to find the stock plants which were apparently the source of the trouble. The Sanford nursery secured its cuttings from one backdoor plant in the city of Sanford, Florida. This plant showed typical symptoms of the disease, that is, vein clearing and zonate ringspots on the leaves. The flowers and other parts of the plant appeared symptomless. The Lake Monroe nursery did not know the source of its infected plants.

SYMPTOMS

First symptoms show on the new leaves as cleared veins. These veins are a cream-yellow to yellow-green color as contrasted with the normal green of an uninfected leaf. In severe infections the midrib plus all or some of the main and lateral veins may be affected (Fig. 1). When infection is less severe, only scattered areas in any part of the leaf may show infection, with some veins or even parts of some veins cleared. Later, vein clearing may be accompanied by some puckering and moderate to slight leaf distortion. There is no apparent reduction in leaf size, although occasionally a smaller than normal leaf showing infection is noted. Finally, as the leaves reach maturity, definite, yellow, partial to complete rings, usually with alternating yellow and green zonations, develop (Fig. 2). Any number from one to many of these zonate ringspots may be scattered over a leaf, corresponding closely to the areas of cleared veins when the growth was younger. It is not uncommon to find a leaf completely or almost completely affected with this condition. In such a case, groups of two or more zonate rings tend to coalesce to give the appearance of one large area of partial and complete rings.

TRANSMISSION STUDIES

In the beginning, three healthy plants of C. thomsoniae were budded with leaf pieces showing symptoms of the zonate ringspot condition. These pieces, 1/4 to 1/2 inch square, were inserted into the stem of the plant following an incision approximately the same size through the epidermis to the cambium tissue. Rubber strips commonly used in budding and grafting were wrapped around the leaf pieces to secure them. In 17 days the initial vein-clearing symptoms, as previously described, appeared. When larger virus-infected plants were available, buds from these infected plants were used as the source of inoculum in later transmission studies. As in the leaf-graft method, first symptoms were noted in 17 to 21 days from the time of budding.

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[1] Plant Pathologist and District Plant Inspector, respectively, Division of Plant Industry, Florida State Department of Agriculture.

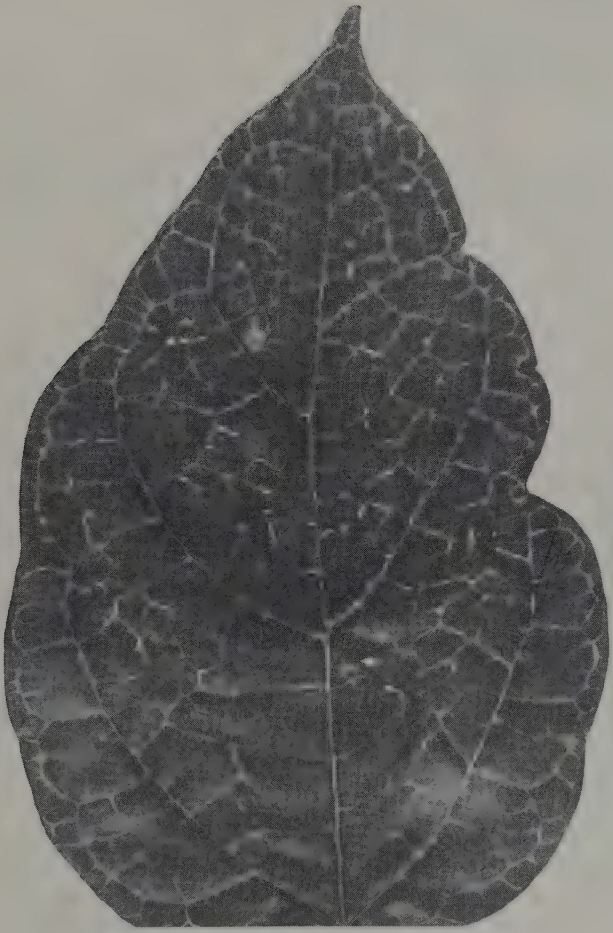

FIGURE 1. Cleared veins, the first symptom of zonate ringspot virus on Clerodendrum thomsoniae.

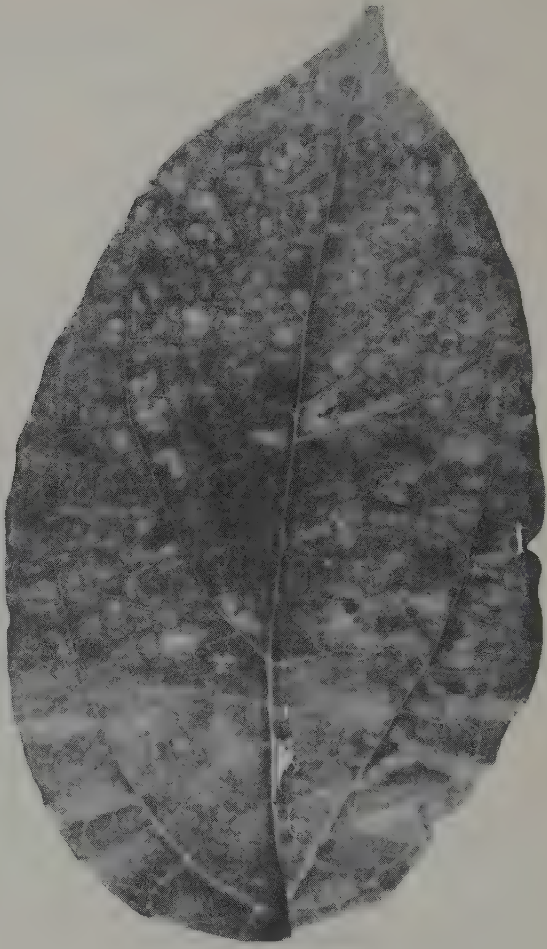

FIGURE 2. Severe zonate ringspot virus symptoms on Clerodendrum thomsoniae.

To date, all attempts to transmit Clerodendrum zonate ringspot virus mechanically from diseased to healthy plants have failed. Freshly expressed juice from infected plants, rubbed on the leaves of healthy C. thomsoniae plants with the use of carborundum powder, gave no symptoms of infection after 60 days.

Host studies among other species in the genus Clerodendrum and in the family Verbenaceae are currently being tested. Plans are to test other plants in this family as they become available.

FLORIDA STATE DEPARTMENT OF AGRICULTURE, WINTER HAVEN, FLORIDA

NEMATODE POPULATIONS AND THE GROWTH OF APPLE TREES[1]

S. E. Tamburo and Robert E. Adams[2]

Abstract

Large numbers of nematodes in the soil from the rhizosphere of apple trees in West Virginia were found to be associated with poor terminal growth of the trees. Additional evidence that this poor growth was associated with high populations of nematodes was obtained in the greenhouse with apple seedlings grown in sand culture, with and without nematodes, and supplied with equal quantities of nutrient solution. The nematodes found in these studies included the genera Rotylenchus, Tylenchus, Pratylenchus, Neotylenchus, Xiphinema, Paratylenchus, and Rhabditis.

INTRODUCTION

In commercial apple orchards, individual trees or small groups of trees are commonly found that make poor growth, while adjacent trees having an identical cultural program and similar soil make satisfactory or even vigorous growth. Frequently this condition is attributed either to localized poor soil drainage or to other vague differences that may be difficult to substantiate or to disprove. The junior author[3] proposed that this condition under certain circumstances may be due to ectoparasitic nematodes in the soil. The purpose of this paper is to report the results of further testing of the hypothesis that injury caused by nematodes is a factor limiting tree growth.

EXPERIMENTS

Preliminary Studies: Orchard trees showing poor terminal growth were examined and compared with adjacent normal trees for two growing seasons to determine whether bacteria, fungi, insects, mechanical damage, or pesticide spray toxicity were more prevalent on the poorly growing trees than on the normal. No differences were found. An examination of the soil profile under normal and affected trees yielded no explanation for the poor growth.

Field Studies: Because of the negative results in the preliminary studies, a systematic evaluation of tree growth and associated nematode populations in the soil about the roots of affected and normal trees was undertaken. Measurements of the annual terminal growth of the trees were made and soil samples from the roots of these trees were taken during January 1958, in orchards situated near Romney, Kearneysville, and Morgantown, West Virginia. One hundred terminals were measured from each of three poorly-growing and three normal Red Delicious trees in the Morgantown and Romney orchards, whereas in the Kearneysville orchard, 100 terminals were measured from each of five normal and five poorly-growing Stayman trees. Five 12-inch soil cores were taken from beneath the canopy of each of the trees measured, and the cores from each tree were thoroughly mixed to form a composite sample. The volume of the sample was measured so that numbers of nematodes in each 50 ml of soil could be calculated. The nematodes were extracted from the total sample by differential sieving through 60-, 200-, and 325-mesh screens[4]. The nematodes collected on the 200- and 325-mesh screens were further separated from soil debris by the Baermann funnel technique, and identified to genus.

The average length of terminals from normal trees was 9.4 inches, and an average of 83 nematodes was found in each 50 ml of soil. The terminals of poorly-growing trees averaged 2 inches in length with an average of 190 nematodes in each 50 ml of soil. The measurements of terminal growth and the average numbers of nematodes found in 50 ml of soil from each tree are reported in Table 1. All soil samples contained nematodes of the genera Pratylenchus,

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[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific paper No. 604.

[2] Respectively, Former Graduate Assistant in Plant Pathology and Associate Plant Pathologist, West Virginia Agricultural Experiment Station, West Virginia University, Morgantown.

[3] Adams, Robert E. 1955. Evidence of injury to deciduous fruit trees by an ectoparasitic nematode (Xiphinema sp.) and a promising control measure. Phytopathology 45: 477-479.

[4] Cobb, N. A. 1918. Estimating the Nema Population of Soil. U. S. Dept. of Agr. Tech Circ. 1: 1-47.

Rotylenchus, *Tylenchus*, and *Rhabditis*. In addition, a species of *Neotylenchus* was found in the samples from Kearneysville, *Xiphinema americanum* in the Romney samples, and *Paratylenchus hamatus* in the Morgantown samples.

Table 1. Length in inches of annual terminal growth of apple trees in three West Virginia orchards and the associated nematode populations in 50 ml of soil from each tree.

Terminal growth in inches		Numbers of nematodes in 50 ml of soil
average[a]	range	
Stayman Winesap, Kearneysville		
13	10-15	74
12	7-15	81
10	9-13	93
9	7-17	92
7	6-11	107
5	1-9	130
3	0.5-6	167
2	1-5	172
2	1-4	198
1	0.5-2	231
Red Delicious, Romney		
12	5-18	61
9	7-15	78
7	5-13	84
2	1-4	191
1	0.5-3	197
1	0.5-2	251
Red Delicious, Morgantown		
9	4-13	91
8	5-14	65
8	7-12	88
2	1-3	130
2	1-4	237
2	1-4	192

[a] The average length of 100 terminals selected at random from each tree.

To emphasize the nature of the relationship found between nematode populations and annual terminal growth, a portion of the data, from Stayman apple trees, is presented in graphic form in Figure 1. The length of terminal growth is negatively correlated with the nematode populations around the roots of the trees.

Greenhouse Studies: Studies in the greenhouse were initiated to test the above hypothesis further. The trees were grown in sand culture to eliminate any variable in the nutrients available to the trees. The inner surfaces of washed and autoclaved 12-inch clay pots were painted with spar varnish. Each pot was equipped with an automatic siphon[5]. Sand, autoclaved at 10 pounds' steam pressure for 1 hour in 10 pound lots, was added to the pots and then thoroughly washed with distilled water. Seeds from Golden Delicious and York Imperial apples were treated with thiram and planted in the sterile sand. Equal quantities of Hoagland's nutrient solution[5] were supplied to the trees in each pot on alternate days. The pots were flushed with distilled water every eighth day. After the seedlings had reached 1 inch in height, they were thinned to 20, equal-sized seedlings per pot. Approximately 200 individuals from the mixed nematode populations, obtained from the field washings, were used to inoculate trees 1 1/2 inches high, growing in sand culture in the greenhouse. Eleven pots were inoculated and 11 were used as controls. A total of 440 trees was used in these experiments. Pots were randomized on the greenhouse benches to correct for variations in light, temperature, and other factors.

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[5] Gallegly, M. E., Jr., and J. C. Walker. 1949. Plant nutrition in relation to disease development. V. Bacterial wilt of tomato. Am. J. Botany 36: 613-623.

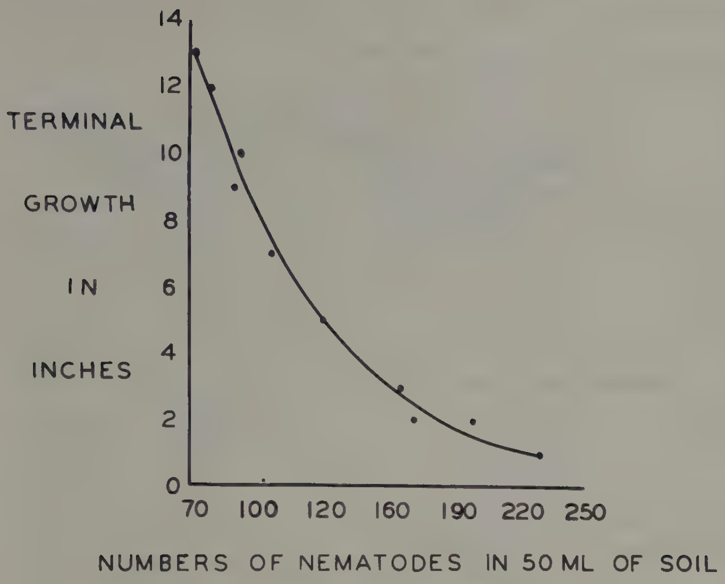

FIGURE 1. The relationship found in one apple orchard between annual terminal growth of Stayman trees and associated nematode populations in the soil.

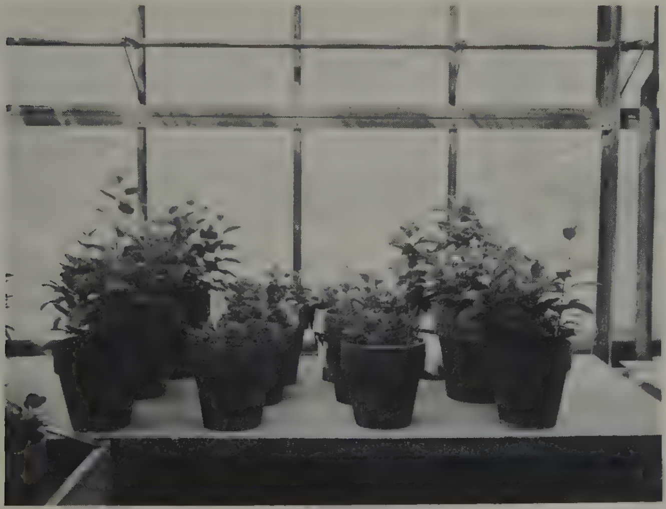

FIGURE 2. The appearance of apple seedlings growing in pots inoculated with nematodes (two center rows), and in non-inoculated pots.

The seedlings were measured after 4 months of growth, and the number of nematodes in 50 ml of sand was determined. The average length of seedlings from inoculated and control pots and numbers of nematodes found in 50-ml samples are reported in Table 2. No nematodes were found in the non-inoculated control pots. The average number of nematodes in inoculated pots was 145 in each 50-ml sample of sand. Representatives of all the genera of nematodes placed in pots were recovered at the end of 4 months, however, *Pratylenchus* and *Neotylenchus* were not recovered in large numbers from either soil or roots. The appearance of trees in inoculated and control pots is shown in Figure 2, and the relative lengths of inoculated and non-inoculated seedlings in Figure 3.

DISCUSSION

The results of both field and greenhouse studies show that poor terminal growth of apple trees is associated with large nematode populations. When mixed populations of nematodes were added to sterile sand in which apple trees were growing, the nematodes increased in numbers. The seedlings in pots to which nematodes were added were shorter when compared with those in the control pots. Which nematode genera were responsible for the poor growth was not determined. However, the damage done to the apple seedlings by the populations from the three different orchards was of equal magnitude, and since all generic representatives reproduced, it seems likely that all contributed to the poor growth of the apple seedlings.

With a perennial plant such as apple, the nematode problem may well be different from that on an annual plant where one nematode species may be primarily responsible for the damage done. These studies indicate that limited growth of apple trees is related to the number but not necessarily the generic structure of the nematode population present, providing the individuals are capable of reproducing on the host.

In the greenhouse studies there were few root hairs on the roots of seedlings in inoculated pots, but root hairs were abundant on roots of seedlings in uninoculated pots. This might partially explain the poor growth observed.

The results of these studies lend additional support to the hypothesis that nematodes occur in damaging populations on the roots of apple trees, and emphasize the importance of an in-

intensive, continuing search for practical control measures.

Table 2. Average length in inches of apple seedlings in nematode-inoculated and uninoculated pots, and average numbers of nematodes in 50 ml of the sand.

Inoculated			Uninoculated		
length in inches	no. of trees	no. of nematodes in 50 ml of sand	length in inches	no. of trees	no. of nematodes in 50 ml of sand
Inoculated with nematodes from Kearneysville					
7.8	80	131	16.7	80	0
9.1		138	16.7		0
8.0		152	11.0		0
7.9		147	17.5		0
Inoculated with nematodes from Romney					
6.6	80	155	17.1	80	0
7.9		138	15.6		0
9.5		114	17.8		0
8.0		120	11.7		0
Inoculated with nematodes from Morgantown					
7.2	60	185	11.0	60	0
8.9		169	17.1		0
7.3		153	21.4		0

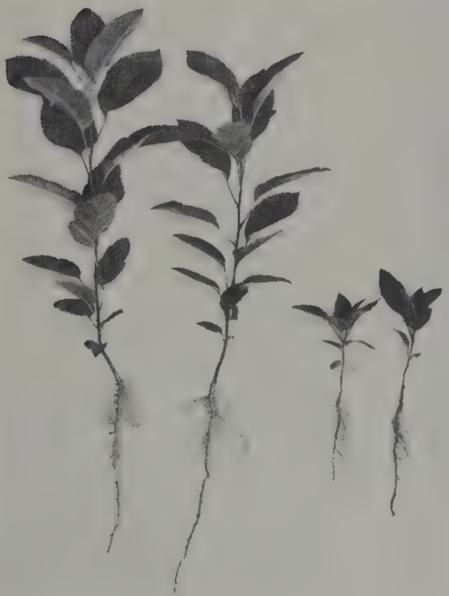

FIGURE 3. Closeup of seedlings grown in inoculated pots (right) and in non-inoculated pots (left).

WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION, MORGANTOWN

A BASAL CANKER DISEASE OF EASTERN WHITE PINE

S. B. Silverborg, J. R. Risley, and A. L. Leaf[1]

Many forest plantations established during the early 1930's were planted without adequate knowledge of optimum site requirements for many species. As a result many of them were early failures while others, after approximately 25 to 30 years, are beginning to decline.

An example of a disease of eastern white pine, Pinus strobus, planted on a shallow, poorly-drained, fragipan soil and resulting in the death of many trees, is reported here for the first time. It was observed in 1956 near the town of DeRuyter in central New York. More recently it has been reported from plantations in southwestern New York.

The DeRuyter plantation, approximately 96 acres in size, is located at an elevation of 1800 feet. It was established on abandoned farm land in 1932 with 3-year-old seedlings spaced 4 feet by 4 feet. The average height and d. b. h. of the trees at the present time is 25 feet and 5 inches, respectively. The plantation was thinned once in 1954.

Description of Cankers: The cankers occur at the base of the trees from ground level to a height of approximately 12 to 24 inches. The first visible symptom is a flattening of the lower trunk and a formation of callus tissue at the margins of the canker (Fig. 1). In young cankers the bark is firmly attached, but later becomes loose, exposing dead sapwood that is stained black (Fig. 2). Cankers frequently develop on opposite faces, resulting in a flattened lower trunk (Fig. 3). The cankers are equally common on all crown classes. Death results from a girdling of the trunk in 2 to 5 years or more.

Soil Description: The soil is classified in the Chippewa series, a poorly-drained soil with a fragipan at a depth of 8 to 12 inches. The soil, derived from late Wisconsin glacial till, contains siltstone and shale fragments throughout the profile. The surface 6 inches of soil is dark gray-brown, silt loam texture, contains 13. 66% organic matter, and has a pH of 3. 9. The A_2 horizon as well as the B_2 fragipan horizon are olive gray-brown color, mottled, have a silt loam texture, and a pH of 4. 0 to 4. 4. The laboratory analyses of the soil involved procedures in Wilde and Voigt (2). The soil has many of the characteristics of poorly-drained sites on which red pine, Pinus resinosa, begins to decline at an early age (1).

Extent of Damage: To determine the extent of damage due to the basal canker disease, a random sample line containing 100 trees was established in the DeRuyter plantation in 1956. At the time that the first observations were made in 1956, 54 trees had basal cankers but were still alive, 41 were healthy, and 5 were dead[2]. Four years later, in 1960, cankers had developed on 14 more trees, only 19 healthy trees remained, and 13 trees were dead. At the present time 2% of the trees on the sample line are dying each year and new infections are appearing on 5. 5% of them each year.

Fungi Involved: Fruiting bodies of Lophium mytilinum Pers. ex Fr., an Ascomycete belonging to the order Hysteriales, were observed on the bark and on the surface of the stained wood. An unidentified brown-colored fungus was also isolated from the wood on the edges of the cankers. Healthy trees were inoculated with cultures of both fungi to determine if they were pathogenic. Results of these tests were negative.

DISCUSSION

From our present knowledge, the basal canker disease appears to be the result of planting eastern white pine, a moderately deep-rooted species, on shallow, poorly-drained fragipan soils. The trees apparently grow normally for approximately 20 to 25 years, but when the roots reach the shallow impervious layer they soon begin to decline. Another possible contributing cause to canker development and subsequent early decline may be root competition between the closely-spaced trees. Since the roots are unable to penetrate the fragipan stratum they grow laterally and compete with their neighbors for soil nutrients.

An early thinning or a wider initial spacing, resulting in less root competition, may delay canker development. However, it is doubtful whether such a site will ever produce more than a short rotation crop of eastern white pine.

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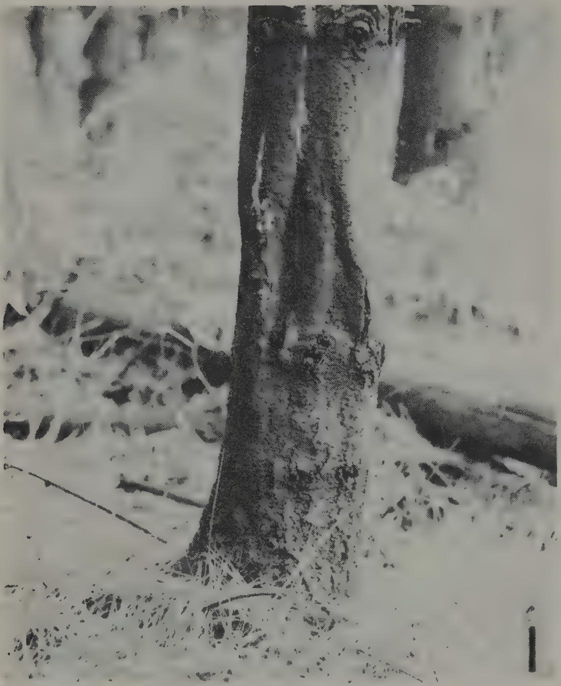
[1] Respectively, Associate Professor, Department of Forest Botany and Pathology, State University College of Forestry at Syracuse University; Forest Biologist, New York State Conservation Department; and Assistant Professor, Department of Silviculture, State University College of Forestry at Syracuse University.

[2] Since the 5 dead trees had basal cankers, death was attributed to the canker.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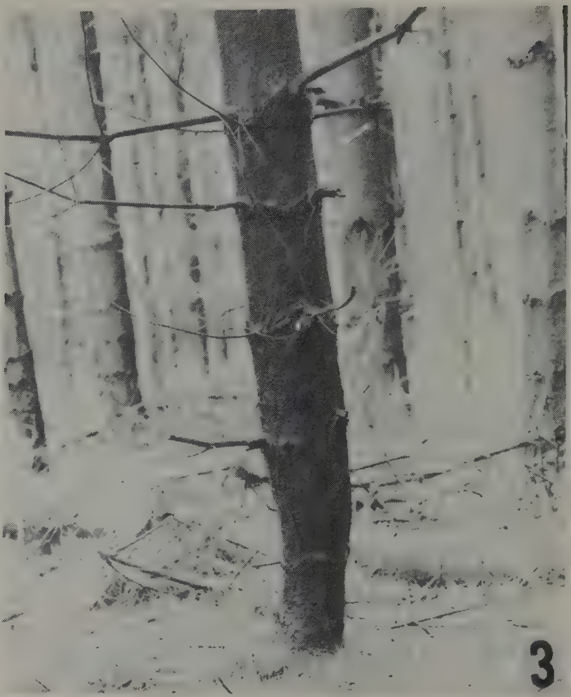
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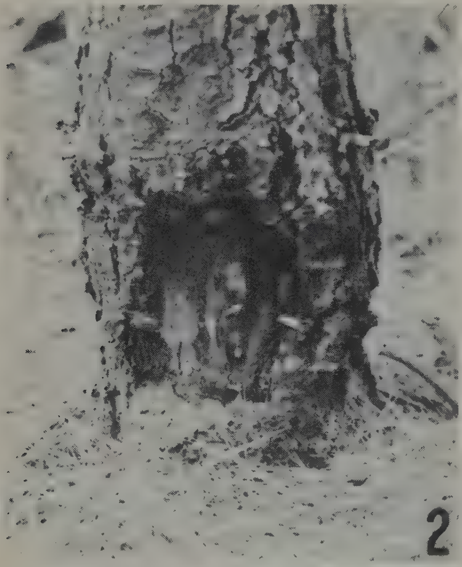
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FIGURE 1. Young basal canker on a 5-inch tree showing a depressed area with firmly attached bark and callus tissue on the margins.

FIGURE 2. Canker on a 6-inch tree. The bark has sloughed off, exposing a dark-stained sapwood.

FIGURE 3. Basal canker on opposite faces of a 6-inch diameter tree, resulting in a constricted base.

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STATE UNIVERSITY COLLEGE OF FORESTRY AT SYRACUSE UNIVERSITY AND NEW YORK STATE CONSERVATION DEPARTMENT

WEAK PEACH, A NEW VIRUS DISEASE OF PEACH

Glenn KenKnight[1]

Summary

An apparently unreported virus was discovered in an Erli-Red-Fre peach tree at Fort Valley, Georgia and named the weak peach virus (WPV). In graft-transmission experiments the virus delayed flowering, foliation, and ripening of fruit of certain peach varieties. Those symptoms reoccurred annually. Slight to severe dieback of branches occurred on some inoculated trees. Foliage on affected trees usually became somewhat chlorotic in midsummer. WPV was readily transmitted by bud, scion, bark, or root grafts. The incubation period of WPV in bearing trees was about 1 to 2 years and as long as 4 years in younger trees. Grafts of scion tissue from two Elberta peach trees, a Prunus angustifolia tree, and several Concord grapevines caused a similar disorder on peach index trees, indicating that the source plants were infected with WPV or a similar virus.

In an attempt to determine whether Prunus ring spot virus was present in a peach-variety test planting at the U. S. Horticultural Field Laboratory, Fort Valley, Georgia, 142 orchard trees were indexed on Lovell peach seedlings in September 1952. The index trees were examined in 1953 for delayed foliation or other symptoms usually associated with the ring spot virus, but none were found (2). In 1954, however, two of three trees whose entire top growth had come from the buds of an Erli-Red-Fre peach source tree showed pronounced delayed foliation and severe dieback of branches. Delayed foliation, but not dieback, reoccurred annually thereafter on the two affected trees. The causal agent of the disorder was later named the weak peach virus (WPV).

Transmission Tests: The two WPV-infected test trees were themselves indexed in May 1954 on 1 Fairhaven, 2 Redcrest, and 2 Prairie Rose peach trees and 2 Lovell seedlings. Each index tree was inoculated with several buds from each of the two WPV-infected trees. Each index tree was flanked on one or both sides by an uninoculated control tree of its variety. Symptoms were first noted on these in 1956 when all seven inoculated trees showed marked delay in foliation as compared with their controls. Severe dieback of terminal growth occurred on some inoculated trees but not on any control tree.

In April 1955, bark patches cut from scions of the two original WPV-infected index trees were grafted on limbs of three 6-year-old Early Hiley peach trees. No symptoms developed on the Early Hiley trees in 1956. In 1957 delayed foliation and severe dieback occurred on two. In 1958 all three flowered and leafed out later than their controls, and two died before the end of summer. No casualties occurred among the controls.

In July 1957, scions from the two Early Hiley trees that showed symptoms were treated in a water bath at 50° C for 0, 2, 5, 10, 15, 20, 25, or 30 minutes. For each time period, four 8-year-old Early Hiley trees were inoculated with three or more bark-patch grafts per tree. The hot-water treatment had no effect on transmission of the virus. In the spring of 1958 the inoculated trees averaged 5 days later in reaching full bloom than the control trees. A part of this planting was retained through the growing season of 1961, and each year the inoculated trees continued to be delayed in flowering, leafing, and maturing fruit. Also, in 1958 and subsequent years the foliage on the inoculated trees became chlorotic in midsummer.

At the time the scions were obtained for the hot-water treatments, soil was taken from the area beneath the branches of the two Early Hiley diseased trees. This soil was used to replace the top soil around nine first-year Early Hiley trees. The soil-treated young trees remained normal.

Host Range Studies: Seedling trees of Prunus hortulana, P. mexicana, P. tomentosa, and P. injucunda inoculated with buds from WPV-infected trees did not develop any recognizable symptoms. However, since both the inoculated and uninoculated seedlings of these species differed from tree to tree in time of flowering and leafing, any slight delaying effect exerted by WPV would not have been noticeable.

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Three old P. mahaleb trees, originating from cuttings, were inoculated with WPV. In the following and succeeding years these trees consistently bloomed about a week later than uninoculated controls.

WPV inoculations of various varieties of P. salicina gave inconclusive results because of confusing symptoms caused by the various diseases that make trees of this species short-lived in central Georgia.

Three first-year peach trees of Early Hiley, Elberta, Hiland, Redcap, Cardinal, and Keystone varieties were inoculated with WPV in 1957. Symptoms appeared on the Early Hiley and Elberta trees in 3 to 4 years, but not on the other varieties in 4 years.

Comparison of WPV and Prunus Ring Spot Virus Symptoms: In a greenhouse test, the behaviors of potted peach trees inoculated with WPV, others inoculated with Prunus ring spot virus (RSV), and uninoculated control trees were observed for 3 months. For the test, Lovell seedlings and Redcap nursery-stock peach trees were inoculated with buds from six RSV sources, two trees of each variety for each source; and six Lovell seedlings and six Redcap peach trees were inoculated with buds from WPV-infected trees. The inoculations, made early in March 1960, produced no differences in the subsequent behaviors of the WPV-inoculated test trees and the controls; whereas the RSV-inoculated test trees developed symptoms characteristic of severe forms of ring spot, including retarded foliation, chlorosis, and necrosis.

In a screenhouse test, the behaviors of trees inoculated with WPV and others inoculated with RSV were observed for 3 years. In April 1957, three third-year Golden Jubilee peach trees were inoculated with buds from a RSV-infected Lambert cherry tree, three others were inoculated with buds from a WPV-infected peach tree, and six uninoculated trees served as controls. Neither virus delayed the onset of spring growth. Bark on the RSV-inoculated trees developed cracks and splits in 1958, whereas bark on the WPV-inoculated trees and the control trees remained smooth. By 1960 the WPV-inoculated trees were conspicuously smaller than any control.

A test conducted out of doors compared the effects of the two viruses on second-year Georgia Belle peach trees. In July 1957, six Georgia Belle peach trees received buds from a RSV-infected Lambert cherry tree, six received buds from a WPV-infected peach tree, and six uninoculated trees served as controls. In 1958 the RSV-inoculated trees showed delayed onset of growth. Bark on the trunk and scaffold limbs cracked and split, and three trees died. The surviving trees tended to recover and thereafter bloomed and leafed at the same time as the controls. The bark of the WPV-inoculated trees remained smooth, but onset of growth was retarded in 1960 and 1961.

DEBILITATING VIRUSES GRAFT--TRANSMITTED FROM VARIOUS SOURCES

The WPV inoculum employed in the graft-transmission experiments described herein was infective as a result of direct or serial passage of the causal virus from a single Erli-Red-Fre orchard tree. It is not known whether the disease caused by WPV spreads naturally. In three instances disorders similar in many respects to WPV disease developed on peach test trees as a result of indexing other plants on the Laboratory grounds. The relation between WPV and the causal agent or agents of the other diseases was not established.

Elberta Peach Trees: A block of 2000 Elberta trees, planted in 1946, was routinely inspected for phony in 1949. One tree that was recorded as phony in July appeared normal in September. Of several scions taken from the tree and rooted in a sand and peat moss mixture, one survived. This grew into an apparently normal cutling tree. In 1953, scions from the cutling tree were grafted on low branches of ten first-year Golden Jubilee trees in a screenhouse. No symptoms were noted on the inoculated trees in the next 3 years. In 1957, however, it became obvious that a gradual stunting of the inoculated trees had occurred. The trunk diameter of each of the inoculated trees was smaller than the average diameter of the controls. Also, the inoculated trees had an excessive number of small terminal twigs. The latter symptom was not noticeable in subsequent years.

In April 1951, new-case phony trees showed symptoms unusually well for so early in the season. However, of the many trees in the Elberta block classed as probably phony, several gave negative reactions to the chemical test for phony (1) and were symptomless in July. Such trees were root- and scion-indexed on Early Hiley trees. No symptoms developed on the test trees in the 2 years they were observed. Further scion-indexing of the source trees was undertaken in February 1954. In one instance scions from a slightly dwarfish but otherwise normal-appearing Elberta tree were grafted on 67 second-year Lovell seedlings, 1 scion per tree. Fifteen uninoculated Lovell seedlings, interspersed in groups of five among the inoculated trees, served as controls. Only ten scions lived, and the trees bearing these scions died in 1955. The remaining 57 inoculated trees appeared dwarfed in 1955 as compared with the control trees. The inoculated trees retained their dwarfish character in subsequent years and showed delayed foliation and midsummer chlorosis.

Wild Plum Trees: In February 1954, ten wild plum trees, *Prunus angustifolia*, which gave negative reactions to the chemical test for phony, were indexed to determine whether any were symptomless carriers of phony virus. Root pieces from each plum tree were grafted to the taproot of first-year Sullivan Elberta peach trees. For each plum tree, six test trees were so grafted. In 1957, the six test trees representing one of the plum trees showed marked dwarfing and a tendency to chlorosis in midsummer. Thereafter these six trees became progressively smaller, less productive, and slower to start growth in spring than the other trees in the wild plum index series.

Concord Grapevines: Cuttings obtained from Concord grapevines infected with Pierce's disease were rooted and grown in a screenhouse. In May 1957, five of these vines were approach-grafted to Golden Jubilee nursery-stock peach trees. In late September 1957, conspicuous bright-yellow dots and lines appeared on lower leaves of four of the five peach trees. The four affected peach trees were approach-grafted to other Golden Jubilee peach trees. Symptoms similar to those caused by WPV appeared 2 to 3 years later. Yellow markings on the leaves were never again noted.

The virus of Concord grape gave results similar to those produced by WPV in the March 1960 greenhouse test. In that test each of six Lovell seedlings and six Redcap peach trees were inoculated with buds from the affected Golden Jubilee peach trees. No symptoms developed on the test trees in the 3 months they were observed.

DISCUSSION

Whether the diseases reported herein occur naturally in commercial peach orchards in the Fort Valley area is unknown. Dwarfish trees more or less affected by dieback and midsummer chlorosis occur rather commonly. From 1954 through 1956 approximately 30 such trees were indexed on varieties susceptible to WPV. An occasional test tree and a few control trees developed symptoms somewhat resembling those caused by WPV. However, at least some of the abnormal trees were affected by root rot caused by *Clitocybe tabescens* (Scop. ex Fr.) Bres. When nursery-stock peach trees were inoculated with root rot by placing Clitocybe-invaded peach stumps in the planting holes, the trees developed symptoms strikingly similar to those caused by WPV. Both disorders caused stunting, dieback, and chlorosis. Under certain conditions both disease delayed the onset of growth in spring.

During 1948-1961 many unthrifty, weak, or dying trees in commercial peach orchards were examined. In some instances root rot or other recognizable diseases were presumed responsible for the condition of the trees. In other instances the cause was obscure. The results of the transmission tests with WPV and the similar or perhaps identical viruses transmitted to peach from grape and wild plum suggest the possibility that debilitating virus infections contribute to the short life of peach trees in this area.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, FORT VALLEY, GEORGIA

STUDIES ON SOME GRAMINICOLOUS SPECIES OF HELMINTHOSPORIUM STORED UNDER OIL[1]

J. L. Peterson and R. A. Cappellini

Summary

After 10 years of storage on agar slants covered with mineral oil, 32 graminicolous species of Helminthosporium were tested for their viability and cultural characteristics. When tested at the end of 7 years, 16 species were viable and 16 were non-viable. The 16 viable species were transferred to three kinds of media, and after 10 years, 12 of them were viable. All the viable isolates which sporulated before storing had lost the ability to form spores; four isolates had noticeable changes in color, three of which had changed after 7 years. No other cultural changes were noted.

INTRODUCTION

The use of an oil seal for the preservation of fungi is currently a common practice in many laboratories. Several reports on the preservation of different fungi under oil have appeared since the first reports by Sherf and others in the 1940's (1, 3, 4). The various genera and species of fungi, however, do not react the same when stored in this way, and we need to know more about the longevity of certain fungi in this kind of storage. In 1958, Cappellini and Haenseler (2) reported on the longevity of isolates of graminicolous species of Helminthosporium when stored under mineral oil for 7 years; of 32 isolates tested, 16 were still viable and 3 of these had lost their ability to sporulate.

This paper reports the longevity and sporulation ability of the same Helminthosporium species and isolates that were used by Cappellini and Haenseler after an additional 3 years of storage.

PROCEDURE

In 1951, 32 graminicolous species of Helminthosporium were subcultured and placed on Difco potato-dextrose agar slants and covered with sterile mineral oil. These tubes were then sealed with "Parafilm" and stored at room temperature. In 1958, 7 years later, the fungi were tested for viability. These same tubes were then retained at room temperature for another 3 years.

In 1961, the 16 isolates that were viable after 7 years were subcultured. Transfers were also made from the other 16 isolates that were reported to be non-viable. Each isolate was placed on Difco potato-dextrose agar, with 0.1% and 1% yeast extract added, and on standard V-8 juice agar. The cultures were held at room temperatures and at 30°C for over 1 month. Growth, color, and sporulation of the cultures were checked periodically. The original description of color and later color changes were taken from Ridgeway's color charts. Three additional attempts were made to recover the fungi when no growth occurred after the initial transfer.

RESULTS AND DISCUSSION

The viability and cultural characteristics of the Helminthosporium isolates after 10 years of storage are given in Table 1. Viability data from an earlier publication (2) on a 7-year storage period is also included in this table for comparison.

The results show that certain Helminthosporium species can survive for 10 years under mineral oil. At the end of 7 years, of the 16 viable isolates, 12 were recovered in this test. The remaining cultures showed no growth after successive transfers on the different media. This was expected with the 16 isolates that failed to grow after 7 years. Two isolates, H. triseptatum and H. tritici-repentis, were transferred three times before growth was noted, which indicates very low vitality. No evident differences in recovery on the different media were noted among the isolates. Of particular significance was the loss of the ability to sporulate by all of the cultures that had sporulated previously. Cultures were held for at least a month and a half for observation on sporulation before discarding. There were slight changes in color noted in four of the surviving cultures and changes were reported for three of these after the 7-year period. Except for the failure to sporulate, no changes in morphological characteristics in the surviving cultures were noted.

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers - the State University, New Brunswick, New Jersey.

Table 1. Recovery and cultural changes of species of Helminthosporium observed after 7 and 10 years of storage under mineral oil.

Species	Recovery[a] (years)		Color change[b] (years)		Sporulation[c] (years)		
	7	10	7	10	0[d]	7	10
H. bromi Died.	-	-			+		
H. buchloës (Ell. & Ev.) Lefebvre & A. G. Johns.	+	+	-	-	+	+	-
H. carbonum Race I Ullstrup	-	-			+		
H. carbonum Race II Ullstrup	+	-	+		+	+	
H. catenarium Drechs.	+	+	+	+	-	-	-
H. cyclops Drechs.	+	+	-	-	+	+	-
H. cynodontis Marig.	+	+	-	-	-	-	-
H. dictyoides Drechs.	+	+	-	-	+	-	-
H. erythrospilum Drechs.	+	+	+	+	+	+	-
H. giganteum Heald & Wolf	-	-			-		
H. gramineum Rabh.	+	-	-		+	-	
H. halodes Drechs.	+	-	-		-	-	
H. leersiae Atk.	-	-			-		
H. leucostylum Drechs.	-	-			+		
H. maydis Nisik. & Miy.	-	-			+		
H. micropus Drechs.	-	-			+		
H. monoceras Drechs.	-	-			+		
H. nodulosum (Berk. & Curt.) Sacc.	+	+	-	-	-	-	-
H. oryzae B. de Haan	-	-			+		
H. ravenelii Curt.	-	-			+		
H. rostratum Drechs.	-	-			+		
H. sacchari (B. de Haan) Butl.	-	-			+		
H. sacchari	+	-	-		+		
H. sorokinianum Sacc. ex Sorokin	-	-			+		
H. setariae Saw.	-	-			+		
H. siccans Drechs.	+	+	-	-	+	+	-
H. sorghicola Lefebvre & Sherwin	-	-			+		
H. teres Sacc.	-	-			+		
H. tetramera McK.	+	+	-	+	+	+	-
H. triseptatum Drechs.	+	+	+	+	+	+	-
H. tritici-repentis Died.	+	+	+	+	-	-	-
H. tritici-vulgaris Nisikado	-	-			-		
H. victoriae Meehan & Murphy	+	-	-	-	+	+	-

[a] + indicates recovery; - indicates non-recovery.

[b] + indicates color change; - indicates no color change.

[c] + indicates sporulation observed; - indicates no sporulation observed.

[d] indicates laboratory cultures which sporulated before storage under oil.

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LEAFHOPPER VECTORS OF PIERCE'S DISEASE VIRUS IN GEORGIA

George H. Kaloostian, H. N. Pollard, and W. F. Turner[1]

Abstract

Three leafhoppers, Homalodisca coagulata (Say), Oncometopia undata (F.), and Cuerna costalis (F.) are reported as vectors of Pierce's disease virus of bunch grapes in Georgia.

A degenerative disease of bunch grapes in the southeastern United States, commonly known as grape decline, has been shown to be caused by the Pierce's disease virus (PDV). Stoner (3) was the first to show a relationship between the disease in Florida and a leafhopper vector, Carneocephala flaviceps (Riley). Crall and Stover (1) confirmed the transmission with the above-named leafhopper and also reported transmissions with Homalodisca spp. (coagulata (Say), insolita (Wlk.), or both), and Oncometopia undata (F.). Hewitt (2) reviewed the history of Pierce's disease in California and the Southeast, and he concluded that PDV is probably native to the Gulf Coast areas of the United States.

The virus-vector relationships of grape decline have been investigated at Fort Valley, Georgia since 1955. In 1955 W. F. Turner and H. N. Pollard initiated a total of 20 transmission tests, using rooted Niagara cuttings purchased from a nursery in Maryland. They were grown in a screenhouse at Fort Valley. The test insects were collected in the field and bagged on PDV-infected Concord grapevines from July 22 to 25 for the virus-acquisition feeding period. After that period the insects were caged on the test plants, 9 to 17 per plant, for a transmission feeding period of 21 to 105 days.

W. B. Hewitt, Plant Pathologist, University of California at Davis, visited the project in September 1957. He diagnosed six cases of Pierce's disease among the 20 transmission tests completed in 1955. The diagnosis was made on the basis of leaf symptoms. The infected vines degenerated during the following summer and died during the winter of 1958-59. The vectors involved were H. coagulata, O. undata, and Cuerna costalis (F.), with 3, 2 and 1 vines inoculated by each species, respectively.

Numerous tests were conducted after 1957, but positive diagnosis of Pierce's disease in the Fort Valley area was complicated because weather conditions were favorable to fungal and bacterial diseases which obliterated symptoms.

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[1] Retired in May 1957.

MEDLAR IS HOST FOR GYMNOSPORANGIUM CLAVIPES

Jerry T. Walker and Ellen F. Earhart

The aecial stage of Gymnosporangium clavipes (Cke. & Pk.) Cke. & Pk. was found in August 1961 on several fruits of Mespilus germanica growing in the Earhart Garden in Mulberry, Indiana. Although it apparently was of only minor destructiveness to the fruit of this rosaceous ornamental, this may be the first report of a rust occurring on medlar in this country. A cluster-cup rust, Gymnosporangium confusum Plowr., was reported some time ago on medlar in Europe and as recently as 1944 in England, therefore it is not surprising to find medlar a host for the native rust organism, G. clavipes.

We are grateful to Dr. Frank D. Kern of the Pennsylvania State University for identifying this species, and to Dr. Clark Rogerson of the New York Botanical Garden and Dr. C. W. Ellett of the Ohio State University for their consultation.

BROOKLYN BOTANIC GARDEN, BROOKLYN, NEW YORK

DISEASES OF RICE NEW TO EL SALVADOR

Oscar Ancalmo and William C. Davis[1]

During the period July to October 1961 two rice (Oryza sativa) diseases not previously reported in El Salvador were identified. The determination that both diseases are new to El Salvador is based on publications by Abrego, et al. (1) and Ancalmo (2).

1. Leaf scald disease of rice, caused by Rhynchosporium oryzae Hashioka & Yokogi[2]. In a field test of four replications the varieties Bluebonnet 50, Nato, Magnolia and Zenith were more severely attacked than Pandhori No. 4 and Asahi.

2. White tip disease of rice, caused by Aphelenchoides besseyi Christie[3]. This disease was found in experimental trials at one location on such varieties as Lacrosse, Zenith, Gulfrose x 253, and some lines introduced from Surinam.

Either or both diseases might become economically important to El Salvador.

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DIRECCION GENERAL DE INVESTIGACIONES AGRONOMICAS, SANTA TECLA, EL SALVADOR, C. A.

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[1] Plant Pathology Department, Dirección General de Investigaciones Agronómicas, Santa Tecla, El Salvador.

[2] Identification of the fungus was kindly made by Mr. Albert S. Muller, University of Florida.

[3] Identification verified by Dr. Vernon C. Perry, University of Florida and Dr. A. Morgan Golden, Nematology Investigations, USDA.

BOOK REVIEW

"HOW TO CONTROL PLANT DISEASES IN HOME AND GARDEN," by Malcolm C. Shurtleff, Iowa State University Press, Ames. viii + 520 pages. 1962. Price $4.95.

This practical book for home gardeners reflects a basic aim of Dr. Malcolm C. Shurtleff's career as a public servant: to make his science useful to anyone with a plant disease problem. During his years as Extension Plant Pathologist with two midwestern universities, Dr. Shurtleff has talked to and written thousands of people about controlling plant diseases. This book represents a more difficult but further effort toward his goal.

How to Control Plant Diseases sets out to help the home gardener answer two questions: What Is It? and What Can I Do About It? These, in fact, are section headings for the heart of the book; the first defines diseases in pictures and words, and the second details disease control measures. Dr. Shurtleff organized his discussion of diseases according to plant parts; foliage diseases and stem diseases are examples. The choice and application of effective chemicals highlights What Can I Do About It?

These two main sections are supported by an introduction that tells the reader how to use the book most effectively, as well as gives him a general orientation in plant pathology. In addition, an extensive fourth section alphabetically lists garden plants and their diseases.

A glossary defines useful terms uncommon to most home gardeners, and an appendix includes information on preparing chemical sprays and dusts and treatment schedules for their use on various types of garden plants. The book is color-indexed along page margins as a quick reference aid to the reader. --PAUL R. MILLER

RESULTS OF 1961 FUNGICIDE AND NEMATOCIDE TESTS

The "Results of 1961 Fungicide and Nematocide Tests" is now available. This report is issued annually by the American Phytopathological Society, Subcommittee on New Fungicide and Nematocide Data. This report serves as a medium for organizing and presenting the summarized results of current fungicide and nematocide testing projects. Much of the information is never otherwise published or made conveniently available. Information on products available for testing, composition of products and their sources are given.

Copies of this report are available at $1.00 per copy when accompanied by a remittance, $1.25 when invoiced and billed. Address orders to A. B. Groves, Winchester Fruit Research Laboratory, 2500 Valley Avenue, Winchester, Virginia. Make remittances payable to the American Phytopathological Society.

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14

Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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(3) ABSTRACTS are requested for all except very short articles.

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so.

(5) LITERATURE REFERENCES should be given in alphabetical order and numbered for citation in the text. Place the year of publication after the author's name. Please check your references carefully since we cannot always do it for you. Be sure that text citations and bibliography agree. Cite number or month for periodicals that are not paged consecutively throughout the volume.

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ACCEPTANCE OF MANUSCRIPTS

The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to twelve double-spaced typed pages, including tables, graphs, and photographs. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases.

Paul R. Miller

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CONTENTS

1. Effect of thawing temperature on urediospores of Puccinia graminis f. sp. tritici frozen in liquid nitrogen
WILLIAM Q. LOEGERING and DANIEL L. HARMON 299

2. Nematode transmission of yellow bud mosaic virus to strawberry
N. W. FRAZIER and A. R. MAGGENTI 303

3. Eyespot, a new foliar disease of bananas caused by Drechslera gigantea
DONALD S. MEREDITH and FRED G. CAMPBELL 305

4. Greenhouse tests using antibiotics to control blister rust on white pine
E. P. Van ARSDEL 306

5. Downy mildew of broccoli in the Pacific Northwest
ARLEN D. DAVISON, et al. 310

6. Fungi associated with gladiolus roots under continuous cropping conditions
CURTIS R. JACKSON 312

7. Role of trichomes in contact transmission of viruses
C. E. YARWOOD 317

8. Botrytis inflorescence blight on American holly in New Jersey
STEPHEN BACHELDER and E. R. ORTON 320

9. New hosts for three Helminthosporium species from corn
ALICE L. ROBERT 321

10. Symptom stability in sweet cherry trees affected by crinkle leaf and deep suture
A. W. HELTON 325

11. Control of plant-parasitic nematodes and weeds in holly nurseries
FRANK A. HAASIS and J. N. SASSER 328

12. Pathogenicity of Stemphylium loti on several blue lupine breeding lines resistant to S. solani and S. botryosum
HOMER D. WELLS, et al. 333

13. Three cowpea mosaic viruses from gladiolus
PHILIP BRIERLEY and FLOYD F. SMITH 335

14. Mechanical transmission of virus from diseased pear trees to herbaceous hosts
HILLEGONDA J. PFAELTZER 338

15. Fomes annosus: A report on the production of conidia in nature and other studies in Virginia
C. L. MORRIS and K. A. KNOX 340

16. Preliminary investigations on biological control of gray mold (Botrytis cinerea) of strawberries
DIBYA DEO BHATT and E. K. VAUGHAN 342

17. Stylet-bearing nematodes associated with white clovers in New Hampshire 1960-1961
TSEH-AN CHEN, et al. 346

18. Powdery mildew on strawberry in Florida
HARRY C. BURNETT and HOWARD Van PELT 348

19. Diverse symptoms in Pelargonium infected with Verticillium
FRANK P. McWHORTER 349

20. Pitting disease of banana on the market
LOUIS BERAHA 354

21. Evidence against seed transmission of green ring mottle virus in mazzard cherry seedlings
R. M. GILMER and K. D. BRASE 356

22. Prevalence of Thielaviopsis basicola in California citrus soils
PETER H. TSAO 357

23. Studies on epidemiology of bacterial angular leafspot on strawberry
B. W. KENNEDY and T. H. KING 360

24. A study of symptoms and control of Phomopsis juniperovora on Arizona cypress
A. T. McDANIEL and C. L. WILSON. 364

25. An effective program for the control of banana mosaic
A. V. ADAM 366

26. Advantages of determining the tolerance of strawberries by grafting with leaves infected with a virus complex
P. W. MILLER 371

27. Occurrence of certain plant diseases in Kentucky in 1961
E. M. JOHNSON, et al. 372

28. Reduction of soil populations of the citrus nematode by the addition of organic materials
R. MANKAU and R. J. MINTEER 375

29. Observations on the succession of fungi on hardwood pulpwood bolts
ALEX L. SHIGO 379

30. A new Phytophthora fruit rot of Achras sapota from India
VASANT GURUNATH RAO, et al. 381

31. Determination of nematode mortality
LAWRENCE M. FENNER 383

32. Possible use of urethane foam for filling cavities in trees
CURTIS MAY 384

33. Correction 384

EFFECT OF THAWING TEMPERATURE ON UREDIOSPORES OF PUCCINIA GRAMINIS F. SP. TRITICI FROZEN IN LIQUID NITROGEN

William Q. Loegering and Daniel L. Harmon[1]

Abstract

Urediospores of Puccinia graminis f. sp. tritici can be frozen in liquid nitrogen without injury to them. Their germinability is restored by thawing in a water bath for 2 to 5 minutes at temperatures in the range of 40° to 50° C. Thawing below 30° does not restore germinability but spores so treated will germinate normally if shortly after thawing they are subjected to 40° to 50°.

INTRODUCTION

Long-time preservation of viable urediospores of Puccinia graminis Pers. f. sp. tritici Eriks. & E. Henn. would be useful in maintaining a type culture collection as well as in handling the fungus in testing procedures and in research work. Urediospores held in the usual refrigerator for several weeks retain sufficient viability to be used in routine inoculation work; however, all viability usually is lost after about a year. Sharp and Smith (4, 5) developed a vacuum-drying method which has made possible the keeping of urediospores in a viable condition for 5 years or more. Loegering, et al. (2) reported that urediospores frozen in liquid nitrogen (-196° C) and thawed at 37° germinate normally. This paper reports studies on the effect of thawing temperature on urediospores frozen in liquid nitrogen.

MATERIALS AND METHODS

Culture 15B-59F of Puccinia graminis f. sp. tritici was used. Urediospores were produced in the greenhouse on 6- to 8-week-old Little Club wheat plants which had been inoculated by hypodermic injection with a suspension of urediospores. The spores were collected by shaking them from the plants onto paper and then screening out the small pieces of sand and debris with a 60-mesh sieve.

About 1 mg of dry spores was placed in 6-inch tubes made from 7-mm borosilicate glass tubing. The tubes were sealed with a cross-fire oxygen-gas torch. These tubes were fastened to wire canes with surgical tape found to be the best material for this purpose. These canes with the tubes attached were placed directly into the liquid-nitrogen refrigerator. When it was desired to thaw the spores, a cane with the attached tubes containing the spores was removed from the refrigerator and plunged immediately into a water bath held at a predetermined temperature.

Percentage germination of the spores was determined on water agar (1). For each treatment two tubes were used, three water agar plates were seeded from each tube, 100 spores were observed on each plate, and the germinated ones were counted. The percentage germination of the fresh spores was determined when the tubes were prepared and a base of 100% was established. The actual percentage germination of treated spores was corrected accordingly and is expressed in Tables 1 to 6 and Figure 1 as adjusted percentage germination. Unless otherwise indicated in the text, the methods described were used.

RESULTS

Spores which had been frozen in liquid nitrogen were thawed for 2 minutes at various temperatures from 20° to 70° C (Table 1). Those thawed at 20° showed practically no germination; those thawed at 30° were very low in germination; those thawed at 32° and 34° were variable in germination not only between tests but between replicates; those thawed at 36° germinated almost normally; those thawed at 38°, 40°, 50°, and 60° germinated as well or better than the checks while those thawed at 70° germinated poorly because of heat damage. Spores were thawed at 40° for periods of 15 seconds to 40 minutes (Table 2). In all cases germination was normal. These data plus experience gained in later work show that the germinability of urediospores of Puccinia graminis f. sp. tritici frozen in liquid nitrogen is

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Table 1. Effect of temperature of thawing on germinability of urediospores of *Puccinia graminis* f. sp. *tritici* frozen in liquid nitrogen.

Test	Thawing temperature in ° C and adjusted % germination									
	20	30	32	34	36	38	40	50	60	70
1	2	11	--	---	---	---	107	107	108	0.6
2	---	29	86	95	100	100	101	---	---	---
3	---	28	11	100	102	119	121	---	---	---
4	0.4	17	78	60	98	100	102	102	103	10

Table 2. Effect of duration of thawing at 40° C on germinability of urediospores of *Puccinia graminis* f. sp. *tritici* frozen in liquid nitrogen.

Test	Time in minutes and adjusted % germination							
	1/4	1/2	1	2	5	15	25	40
1	101	106	108	109	106	106	---	---
2	---	---	---	104	---	104	105	106

Table 3. Effect of thawing at four temperatures for 5 minutes and a following treatment at higher temperatures on germinability of urediospores of *Puccinia graminis* f. sp. *tritici* frozen in liquid nitrogen.

Subsequent treatment	Thawing temperature in ° C and adjusted % germination			
	-2°	0°	20°	40°
Air 18° C	0.2	0.2	0.9	105.0
Water bath 20° C	0.3	2.7	---	---
Water bath 40° C	95.0	95.0	105.0	---

Table 4. Effect of slow heating from 23° C to various temperatures on germinability of urediospores of *Puccinia graminis* f. sp. *tritici* frozen in liquid nitrogen.

Thawing temperature (° C)	Alternate temperature (° C)	Heating time (minutes)	Adjusted germination (%)
23	--	--	0.4
23	30	16	0
23	34	30	9
23	37	42	25
23	40	57	61
40	--	--	104

Table 5. Effect of the interval between thawing at 20° C and treatment at 40° on germinability of urediospores of *Puccinia graminis* f. sp. *tritici* frozen in liquid nitrogen.

Thawing temperature (° C)	Following treatment (° C)	Time interval[a] and adjusted % germination						
		1 min.	15 min.	30 min.	1 hr.	3 hrs.	1 day	1 week
20°	None	0	0.4	0.4	0	0	0	0
20°	40°	106	94	104	106	32	18	0
40°	None	107	106	107	107	107	104	94

[a] Time interval from thawing at 20° C to following treatment and germination.

Table 6. Effect of type of container, thawing temperature, and time held at thawing temperature on germinability of large masses of urediospores of Puccinia graminis f. sp. tritici frozen in liquid nitrogen.

Thawing temperature (° C)	Time of heating (minutes)	Container[a] and adjusted % germination		
		bulb	7-mm tube	5-mm tube
40°	3	67	93	98
40°	8	82	94	96
50°	3	95	93	99
50°	8	100	95	98

[a] The bulb was 1 cm in diameter and contained 0.4 g spores; the 7-mm tube contained 0.2 g spores; and the 5-mm tube 0.1 g spores.

always restored if they are thawed at temperatures between 40° and 50° for 2 to 5 minutes.

When spores in tubes were thawed at 20° C for 2 minutes and then placed at 40° for 2 minutes, germination was normal. A number of experiments were made to investigate this phenomenon. The design of the test, which was repeated more that 10 times, was as follows: treatment 1) spores thawed at 20° for 2 minutes and seeded on water agar; treatment 2) spores thawed at 20° for 2 minutes, placed at 40° for 2 minutes, and seeded on water agar; treatment 3) spores thawed at 40° for 2 minutes and seeded on water agar. The results in all experiments were consistent, 0 to trace germination for treatment 1 and 100% or better germination for treatments 2 and 3. Further studies were made on spores thawed at -2°, 0°, and 20°, after which they were further warmed to room temperature or in a water bath to 20° or 40° (Table 3). Spores treated at 40° germinated normally regardless of the previous thawing treatment but those initially thawed at -2° and 0° were somewhat lower in germination than those thawed at 20° and 40°. In a different test, spores were frozen and thawed six times at intervals of 15 minutes without reducing germinability. The effect of rate of heating was determined as follows: Spores were thawed at 23° C and then slowly heated to various temperatures over a period of time (Table 4). Heating the spores from 23° to 40° over a period of 57 minutes restored the germinability of the spores to only 61%. In a second test spores were thawed at 20° and heated to 40° over a period of 65 minutes. These germinated 75% compared with 0 and 104 for the checks. It was also found that reactivation of spores thawed at 20° must be done shortly after thawing as indicated by the following test: Spores were thawed at 20° and left at room temperature for varying periods before treating at 40° (Table 5). Such spores germinated normally if the 40° treatment was given within 1 hour; after 3 hours the germination was 32%; after one day 18%; after one week 0% while spores thawed at 40° and left at room temperature for the same lengths of time germinated normally. These results indicate that the rate or number of times water in the spores change from the solid to liquid state or vice versa results in little if any damage but that freezing in liquid nitrogen inactivates some mechanism in the spores which is reactivated only by a heat shock at 40° or higher.

For routine work it would be useful if quantities of spores in the range of 0.1 to 0.5 g could be held in liquid nitrogen until needed. Three types of containers

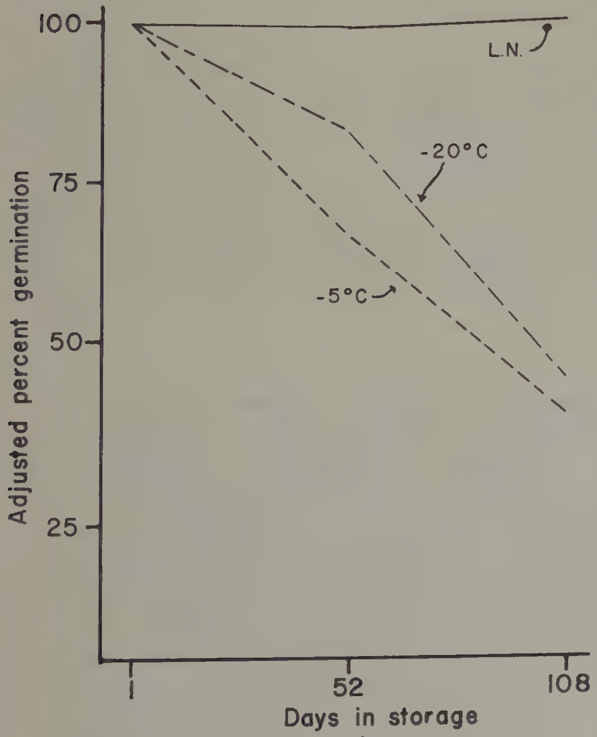

FIGURE 1. Adjusted percentage germination of urediospores of Puccinia graminis f. sp. tritici frozen at -5° C, - 20°, and in liquid nitrogen for 52 and 108 days.

were tried: a bulb 1 cm in diameter containing 0.4 g of spores; a 7-mm tube with 0.2 g spores; and a 5-mm tube with 0.1 g of spores. Four sets of these were frozen in liquid nitrogen and then thawed for 3 and 8 minutes at 40° and 50° C. Spores of each treatment were used to inoculate seedling plants of Little Club wheat. Heavy infection resulted and no differences were evident. The lowest germination (67%) (Table 6) on water agar was by spores from a bulb thawed at 40° for 3 minutes and the highest germinations were of spores from 5-mm tubes regardless of thawing temperature and those thawed at 50° for 8 minutes regardless of container. The lower germinability of the spores from the bulb thawed at 40° for 3 minutes may have resulted from slow heating of the spores in the center of the large mass due to insulation. In a second test with large quantities of spores, 0.1 g of spores was frozen in 5-mm tubes. These were thawed at 20°, 40°, 45°, 50°, and 55° for 5 minutes after 2-months' storage. Adjusted germination was 0, 99, 103, 101, and 86%, respectively. The spores were used to inoculate wheat seedlings. No infection was obtained with spores thawed at 20°; good infection with those thawed at 40°, 45°, and 50°; and fair infection with those thawed at 55°. It appears, therefore, that storage of quantities of urediospores in liquid nitrogen is practical provided they are stored in tubes of small diameter and thawed at 45° C ± 5° before use.

The possibility of storage in deep freeze was investigated. Urediospores were frozen in liquid nitrogen and then removed to -60°, -20° and -5° C. Others were frozen directly at these temperatures. Germinations were made 19 days after freezing, and it was clear that storage in liquid nitrogen was superior to the other six treatments. In a second test of deep freeze storage, spores were stored at 20°, at -5°, and in liquid nitrogen for various periods up to 108 days (Fig. 1). It was clear that storage in liquid nitrogen did not reduce the germinability of the spores whereas storage at -20° and -5° resulted in considerable decrease in germinability.

DISCUSSION

The results demonstrate that freezing urediospores of P. graminis f. sp. tritici in liquid nitrogen does not kill them but does make them "dormant." This "dormancy" can be reversed by subjecting the spores for a few minutes to temperatures between about 36° and 60° C. Spores thawed at 30° or below will not germinate unless given a heat shock at the higher temperatures within about an hour. This phenomenon has not been previously observed in extensive studies of the effect of freezing on biological materials (3). Slow heating from 20° to 40° is not so effective as placing the spores at 40° directly. Large masses of spores do not respond to the heat treatment as well as small masses. A possible explanation is that the spores in the center of the mass are not heated high enough or quickly enough because of the insulation effect of the spore mass.

The possibilities of long-time storage of urediospores of P. graminis f. sp. tritici, and possibly of other rust fungi, in liquid nitrogen are apparent. Such storage will be useful for maintaining type-culture collections and in routine testing and research studies. The length of time spores can be kept in liquid-nitrogen storage is under study (2).

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

NEMATODE TRANSMISSION OF YELLOW BUD MOSAIC VIRUS TO STRAWBERRY

N. W. Frazier and A. R. Maggenti[1]

Abstract

Peach yellow bud mosaic virus was transmitted to healthy Fragaria vesca by populations of Xiphinema americanum, which also may have contained a small number of an unidentified species of Xiphinema. The two species of Xiphinema and virus infection in wild host plants were more common in heavier types of soil than lighter soils verging toward pure sand.

The nematode Xiphinema americanum Cobb, 1913 was first implicated as a vector of yellow bud mosaic virus (YBV) in peach by Breece and Hart (1). Although the virus is known to infect peach and other rosaceous crop hosts, primarily in the interior regions of California, it has also been found along the coast in a few species of wild herbaceous plants including the beach strawberry Fragaria chiloensis (2).

To obtain information on the role of nematodes as vectors of the virus, soil samples were taken from several coastal localities coincident with the plant sampling for virus assay reported by Frazier, et al. (2). Erigeron glaucus appeared to be the most consistently infected of the wild hosts sampled (2). Soil from around this species was sampled in all localities and soil from around other plant species was also usually taken.

The relative soil types, incidences of virus infection in the wild plant hosts (2), methods of testing the soil samples, and the results of the tests varied with locality and may be treated as three groups:

A. Muir Beach; high incidence of natural infection; heavy clay soil; no initial examination for nematodes made. Twenty-four 4-inch plastic pots were planted to cucumber, cowpea and F. vesca strawberry indicator plants and held in a glasshouse. After 4 months no Xiphinema were present although other nematodes were. No YBV symptoms developed in the test plants nor was virus detected in these roots by mechanical inoculation to cucumber or cowpea.

B. 1) Montara; incidence of natural infection nil; sand. 2) San Francisco Beach; incidence of natural infection very low; sand. 3) Stinson Beach; incidence of natural infection nil; light gravelly clay. All soil samples were washed and about 150 Rotylenchus spp. were recovered from Montara, 150 Heterodera spp. from San Francisco Beach, and 50 Heliocotylenchus spp. and 100 X. americanum from Stinson Beach. The nematodes were divided separately into 9 lots of about 50 each and poured with wash water into depressions made in the soil near the crown of a strawberry indicator plant in a 4-inch clay pot. No YBV symptoms developed on any plant and no mechanical assays of roots were made. After 5 months in a glasshouse only three Rotylenchus spp. were recovered.

C. Noyo and Russian Gulch; high incidence of natural infection; variable loam soils. Eighteen soil samples were collected and subsequently divided into two portions: one to be wet screened for nematodes (about 100 cc of soil) and the other to be potted and planted with strawberries. The washed samples were examined for nematodes and then poured into depressions in the soil near the crown of a strawberry indicator plant in a 4-inch clay pot and incubated in a glasshouse. Thirteen of the samples contained variable numbers of X. americanum. In two of the 13 samples small numbers of a second undescribed larger species of Xiphinema were also present. No symptoms of YBV developed over a period of 5 months, at the end of which time virtually no Xiphinema remained in any of the pots. Roots of the strawberry plants were not assayed mechanically.

The remainders of the 18 soil samples were combined into five sample groups and enough sterile greenhouse potting soil added to each to fill a 12-inch clay pot. A healthy strawberry indicator mother plant was set in each and several daughter plants, still attached by stolons to the mother plant, allowed to root. Because the mother and daughter plants were connected by living tissue they can be considered to represent only a single test plant although providing many potential sites of infection. A healthy non-runnering strawberry indicator plant was also set in one of the pots and a YBV infected inoculum plant was set in three pots. After planting the pots were held out of doors under a screen cage on the ground. After 5 months about 100 cc of soil from each pot was examined for residual nematode populations.

The relative number of Xiphinema spp. in the preliminary examination, the planting arrangement, virus transmission results, and the number of residual Xiphinema spp. in the second examination were:

Group 1. Few X. americanum; 1 healthy strawberry; no virus transmission; 2 X. americanum.

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Group 2. Many X. americanum, and few Xiphinema sp.; 1 healthy strawberry; no virus transmission; 40 X. americanum.

Group 3. No Xiphinema; 1 healthy strawberry and 1 diseased E. glaucus; no virus transmission; 1 X. americanum.

Group 4. Many X. americanum; 1 healthy and 1 diseased strawberry; virus transmitted; 20 X. americanum.

Group 5. Many X. americanum, few Xiphinema sp.; 2 healthy and 1 diseased strawberry; virus transmitted to both healthy plants; 15 X. americanum.

In the sample groups four and five symptoms of infection first appeared 4 and 5 months after planting, respectively, in a daughter plant rooted proximal to the diseased inoculum plant, suggesting that the virus was not transmitted by naturally infective nematodes but was first acquired from the virus source plant. The non-runnering strawberry plant in group 5 developed symptoms after 12 months. Over the 12-month period all of the healthy plants in both groups became infected and it is probable that some represented separate infections since the stolon connections became broken during the course of time. The virus appeared to be transmitted only in the presence of a diseased source plant and with substantial initial and residual populations of X. americanum or when Xiphinema sp. was present. Although the undescribed Xiphinema sp. was not observed in the second examination for nematodes it subsequently was found to be present in group 4 as detailed below.

Two months after the appearance of YBV symptoms in group sample 4 all the soil was washed and about 2800 Xiphinema (adults and larvae) were recovered by hand picking. Both species were found, predominantly X. americanum but also a very small number of X. sp. which were not segregated because differences in larval forms could not be detected. The total population was divided into 28 lots of about 100 nematodes each and used to inoculate the soil in the following series of potted plants which were held in a glasshouse for the 8-month duration of the test except for a 6-week cold-rest period out of doors during February and March.

A). Six 6-inch pots containing 2 healthy strawberry and 2 healthy Chenopodium amaranticolor plants;

B). Six 6-inch pots containing 1 diseased and 2 healthy strawberry and 2 healthy C. amaranticolor plants;

C). Sixteen 3-inch pots each with a single healthy strawberry plant.

Twelve plants without nematodes served as controls.

Virus infection occurred only in four plants in series C, in pots to which nematodes had been added, with symptoms appearing 1 1/2, 2 1/2, and, after the cold-rest period, 6 and 7 months after inoculation. No attempts were made to recover virus by mechanical inoculations from roots of plants not showing leaf symptoms, but in these four and the previous three instances of virus transmission to strawberry by nematodes such infection was confirmed by mechanical transmission from the strawberry to cucumber and cowpea.

DISCUSSION

The results indicate that one or more species of Xiphinema may transmit yellow bud mosaic virus and to this extent they agree with the work of Breece and Hart (1). It is probable that X. americanum was the vector in these tests because it represented almost the entire observed population of Xiphinema. If such were the case, then, under the conditions of the tests, it seemed to be an inefficient vector. If Xiphinema sp. rather than X. americanum were an efficient vector, then all observed cases of transmission might satisfactorily be ascribed to it since it was present in all populations where virus transmission resulted.

The presence of Xiphinema and YBV-infected wild hosts in heavier soils and their absence in the lighter soils verging toward beach sand supports the implication that Xiphinema is a vector and provides a favorable natural situation for further investigations.

It is also interesting that two species of Xiphinema were observed in the coastal soil samples, but not X. index, which is a demonstrated vector of grape fanleaf virus.

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EYESPOT, A NEW FOLIAR DISEASE OF BANANAS CAUSED BY DRECHSLERA GIGANTEA

Donald S. Meredith and Fred G. Campbell[1]

Drechslera gigantea (Heald & Wolf) Ito (syn. Helminthosporium giganteum Heald & Wolf) causes zonate eyespot of several grasses in central and southern latitudes of the United States[2,3]. Recent investigations in Jamaica have shown that this fungus is the cause of a previously undescribed foliar disease of bananas. Mature lesions are up to 16 mm long and 6 mm wide. The center of the spot is dull-white or greyish and surrounded by a dark brown margin (Fig. 1). It is proposed to refer to the disease as eyespot of bananas.

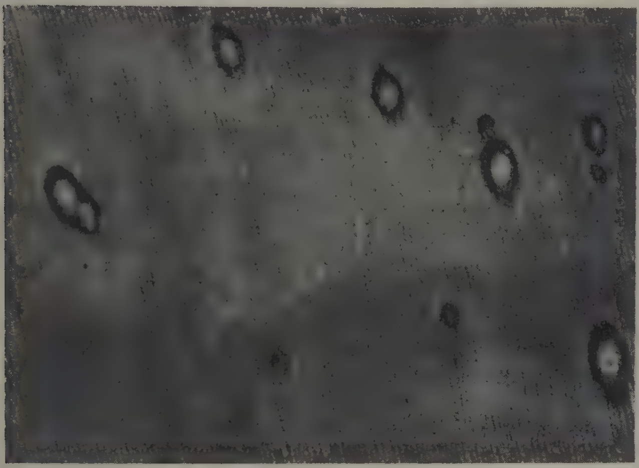

FIGURE 1. Eyespot disease of banana leaves caused by D. gigantea (approx. X 1 1/2).

So far as the authors are aware, this is the first record of D. gigantea for Jamaica and for bananas. The evidence suggests that inoculum for infection of bananas originates on various grasses, particularly Bermuda grass (Cynodon dactylon). Spore trapping studies have shown that conidia of D. gigantea, large as they are (up to 500μ long), become air-borne.

The infection process on bananas is similar to that on grasses, as described by Drechsler[3]. At each end of the conidium, 1 to 4 germ tubes grow out and effect penetration of the host. A detailed account of infection biology is to be published later.

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8, 384

GREENHOUSE TESTS USING ANTIBIOTICS TO CONTROL BLISTER RUST ON WHITE PINE

E. P. Van Arsdel[1]

Abstract

Preliminary results of foliage sprays on cankered (graft transfers) trees indicated that cycloheximide could be transported in the tree, that it could kill cankers by means of foliage sprays on new shoot and needle growth (not hardened off), that sprays on old-growth needles were not effective on small trees, and that phytotoxic effects were serious in foliage sprays at 100 ppm on new shoots (flush growth). Oil sprays were most effective. Phytostreptin discolored cankers and had some effect, but the blister rust continued growth. Purified phytoactin in ethyl alcohol and water was more promising because it had a fungistatic as well as a great discoloring effect.

INTRODUCTION

Antibiotics have been effective as eradicant fungicides on blister rust in white pines. The most notable work has been that of Virgil D. Moss in eradicating the blister rust fungus (Cronartium ribicola Fischer) in cankers on western white pine (Pinus monticola) (1, 2). This paper presents some preliminary work on three antibiotics tested by treating patch-graft transfers of cankers under controlled conditions in the greenhouse in 1957 and 1958 (3).

MATERIALS AND METHODS

Twelve groups of five 4-year-old potted white pines (Pinus strobus) with two established patch-graft transfers of the blister rust fungus on the stem of each were used to test the effects of the antibiotics on the trees and the fungus. Rust sources were from secondary and tertiary transfers of one Iowa County, one Marathon County, and one Vilas County, Wisconsin blister rust cankers. The final transfers of fungus to the test trees were made November 9 to December 3, 1957. Treatments were made on February 26-28, 1958.

Tree growth was controlled by temperature and day-length treatments so that the trees used were in the desired stages of growth, with new shoots with soft needles (flush growth) used in some trials, all hardened off (1-year-old) needles used in some, and mixed stages used in other tests. Trees were incubated for the first 2 months in greenhouses at 60° F (16° C) nights and 68° F (20° C) days.

FIGURE 1. Patch-graft canker of blister rust showing effect of 100 ppm cycloheximide in oil on canker after 2 months. Center of canker is dark brown, edges are bright yellow.

FIGURE 2. Patch-graft canker of blister rust showing normal canker after same period as treated canker in Figure 1. Color is light yellow-green with indefinite edge.

FIGURE 3. Severe injury to new growth from spray treatment with cycloheximide in oil. The tree lived and the rust was killed.

FIGURE 4. Healthy greenhouse-grown white pine shoot from untreated check. Photograph taken at the same time as the one in Figure 3.

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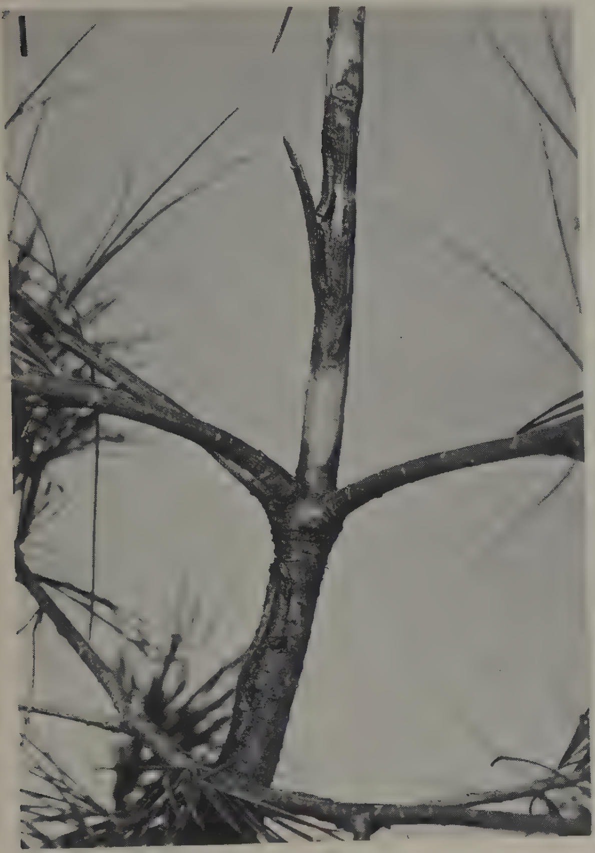
[1] Plant Pathologist, U. S. Forest Service, Lake States Forest Experiment Station, stationed in the Department of Plant Pathology, University of Wisconsin, Madison. Research is a result of cooperation between the Lake States Forest Experiment Station, the Wisconsin Agricultural Experiment Station, and chemical companies who have contributed tested compounds. Cycloheximide was provided by the Upjohn Company, phytostreptin and phytoactin by the Pabst Laboratories, Triton B-1956 spreader sticker by Rohm and Haas Company, and Sovospray S/V 100 by the Standard Oil Company of Indiana. Photographs are by Eugene Herrling, Instructor, Department of Plant Pathology, University of Wisconsin.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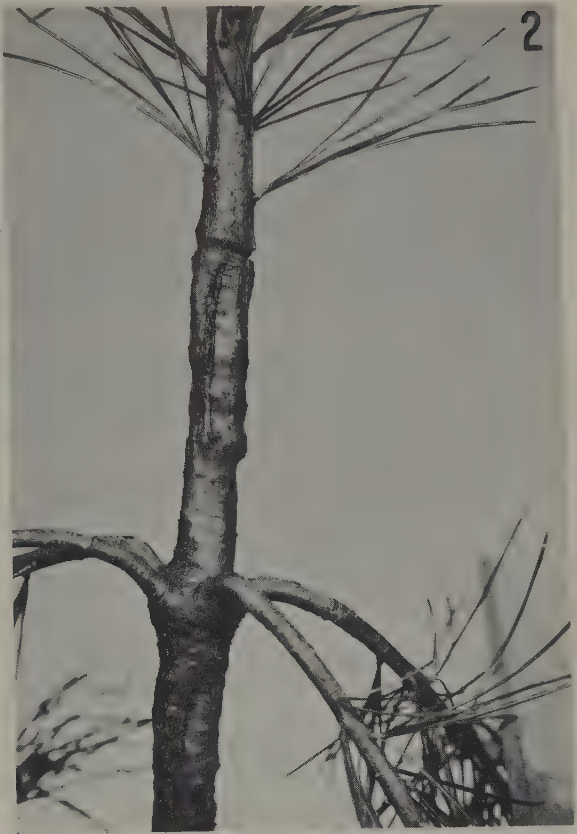
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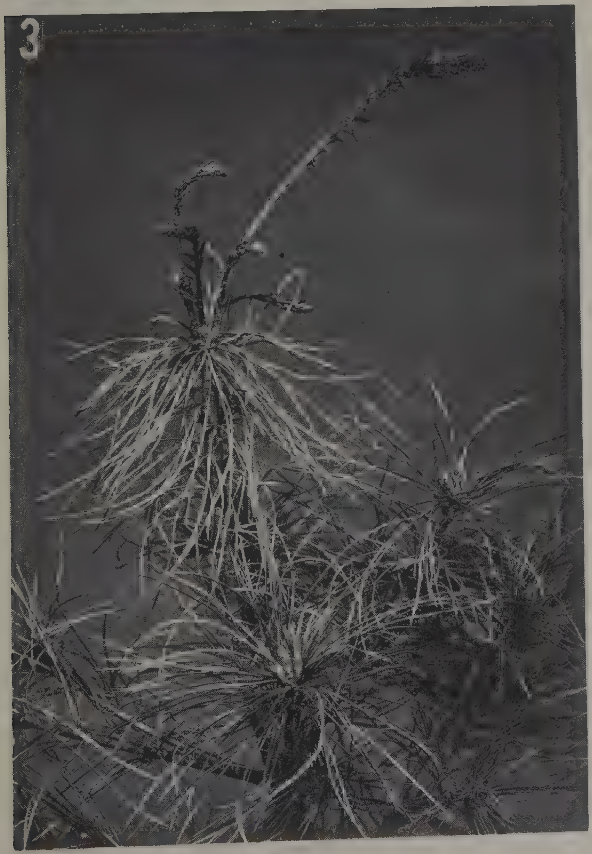
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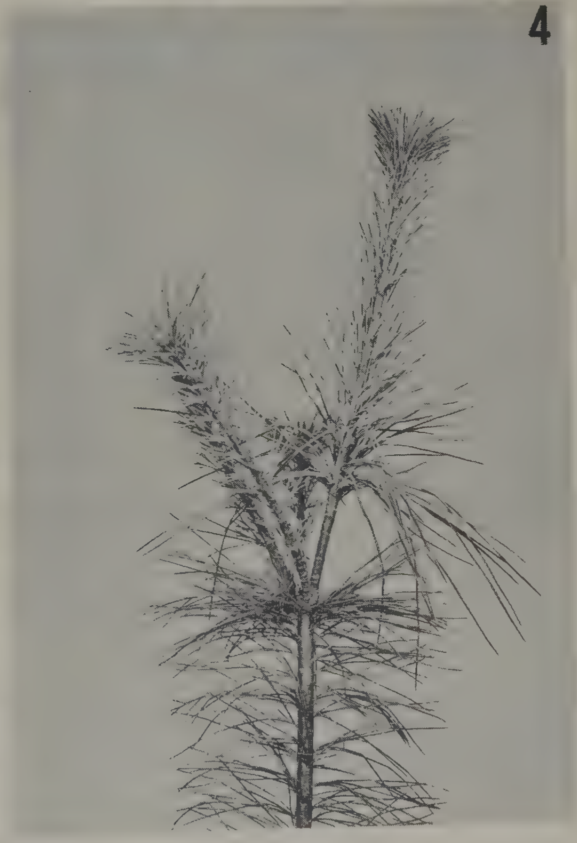
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(See legends on opposite page)

Table 1. Antibiotic treatments and observed results on graft-transferred blister rust cankers on sixty 4-year-old white pine.

Treatment number	Pine growth stage	New shoot length	New needle length	Treatment[a]	Chemical	Carrier	Observations of rust						
							April 22, 1958		January 12, 1959			June 15, 1959	
							Canker color[b]	Foliage injury[c]	Percent of cankers killed	Percent of cankers retarded	Foliage injury c	Average length of growing cankers	Trees killed by rust
		cm.	mm.									mm.	Percent
A	Soft shoot	20-25	25-50	Foliage spray	100 p.p.m. cycloheximide	S/V 100 Sovospray oil	4	5	80	20	6	0	20
B	Old growth	none	none	Foliage spray	100 p.p.m. cycloheximide	S/V 100 Sovospray oil	2	3	0	0	0	94	20
C	Soft shoot	15-20	20-75	Foliage spray	none	S/V 100 Sovospray oil	0	1	0	0	0	91	20
D	Soft shoot	15-20	20-75	Foliage spray	100 p.p.m. cycloheximide	25-percent glycerin in water	3	4	60	40	3	177	0
E	Old growth	none	none	Foliage spray	100 p.p.m. cycloheximide	25-percent glycerin in water	4	3	25	0	3	120	20
F	Soft shoot SNO[d]	8-15	0-25	Foliage spray	none	25-percent glycerin in water	0	0	0	0	0	268	20
G	Soft shoot SNO[d]	10-15	0-25	Foliage spray	100 p.p.m. cycloheximide	Water + Triton B-1956 sticker	4	5	25	0	3	206	20
H	Variable	0-6	0	Foliage spray	100 p.p.m. cycloheximide	Water + Triton B-1956 sticker	4	3	50	0	0	171	0
I	Soft shoot	15-20	15	Foliage spray	none	Water + Triton B-1956 sticker	0	0	0	0	0	193	20
J	Variable	0-25	0-25	Foliage and canker spray	100 p.p.m. phytostreptin	25-percent ethyl alcohol in water	1	0	0	0	0	173	20
K	Variable	0-25	0-25	Foliage and canker spray	100 p.p.m. phytoactin	25-percent ethyl alcohol in water	1	0	0	100	0	250	20
L	Variable	0-25	0-25	none	none	none	0	0	0	0	0	155	20

[a] In treatments "A" through "I" cankers were wrapped in Saran Wrap so that no chemical touched the canker. In treatments "J" and "K" the whole tree, including foliage and the canker, was sprayed. Treatment "L" was left unsprayed as a check.

[b] Canker color code describing appearance of cankers: 0 - normal color of healthy canker; 1 - canker discolored to brighter yellow and orange hues; 2 - canker with bright discolorations and brown necrotic spots; 3 - canker with bright discolorations and large brown necrotic patches (Fig. 1); 4 - canker with bright or brown discolorations, or with large brown patches--canker shriveled and sunken.

[c] Foliage injury code for new growth is as follows: 0 - no injury; 1 - slight yellowing; 2 - severe yellowing; 3 - slight browning of needle ends; 4 - browning of most of needle, but needles green at base; 5 - needles and young stem brown; 6 - all new growth brown, shriveled, and dead.

[d] "SNO" (Sheath Not Open) means the sheath that encloses emerging young needles had not opened to expose needle tissue yet.

TREATMENTS TESTED

The treatments applied and the methods of application are presented in Table 1. Since different types of observations were made on different reading dates, summarized observations are listed for different observation dates.

RESULTS

After 2 months, cankers were an abnormally bright yellow on trees treated with phytostreptin and a brighter yellow on most trees sprayed on the new shoots with phytoactin and cycloheximide (Fig. 1). Figure 2 shows a normal blister rust canker for comparison. With cycloheximide, best success was in the oil spray, but Triton B-1956 in water was also an effective carrier.

After 11 months, cankers treated with phytostreptin were as long as untreated checks; those treated with phytoactin were still bright yellow and had not grown since the 2-month check; cycloheximide-treated trees had dark-brown, dead-looking cankers on those trees treated with oil on new growth. Later observations showed a resurgence of growth in cankers treated with the purified phytoactin.

Both chemical effects on the canker and phytotoxicity on pine foliage indicated the greatest chemical uptake was through the soft new candles of growth with pine needles 1/4 to 3/4 elongated. Seemingly almost no chemical was taken up into the trees through 1-year-old, hardened-off pine needles. In the cycloheximide treatments a reduced proportion of the cankers appeared necrotic with the Triton B-1956 sticker and the 25% glycerin in oil. Trees treated in the hardened-off, no-new-shoot stage showed almost no effect.

Foliage and shoot injury were moderate to severe in new shoot treatments with cycloheximide in the oil carrier (Fig. 3). Figure 4 shows uninjured growth on an equal-aged young shoot for comparison. Oil alone did not produce similar toxicity. Lighter injury occurred with the spreader-sticker and glycerin solution carriers. Foliage injury did not occur with phytostreptin or phytoactin.

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DOWNY MILDEW OF BROCCOLI IN THE PACIFIC NORTHWEST

Arlen D. Davison, Edward K. Vaughan[1], and H. Robert Hikida[2]

Downy mildew, Peronospora parasitica Fr., has been a major disease of broccoli in the Pacific Northwest since 1951 when the variety Waltham 29 was introduced. Since 1954, Northwest Waltham, a selection of Waltham 29, has been grown commercially and damaged severely. The primary loss is the result of infection of the broccoli "head" which often is discolored externally and internally (Figs. 1 and 2). Natti, et al.[3] reported observing the same condition in New York.

EXPERIMENTS AND RESULTS

A number of chemicals have been tested during the past several years in an attempt to develop a satisfactory control of the disease. The incidence of downy mildew was generally moderate to slight during the period of these experiments. In 1957 incidence of mildew was severe for a short time prior to a period of hot weather which retarded disease development for the remainder of the season. The chemicals tested and the degree of control are listed in Table 1.

Table 1. Chemicals tested for the control of downy mildew of broccoli.

Chemical	Rate of active chemical/A.	Number of applications	Type of application	Control
Mylone[e]	200 lb	1	soil[d]	none
	300 lb	1	do.	do.
Maneb (Dithane M-22)	40 lb			
	3.5% dust	6	dust	do.
Maneb (Dithane M-22)	3 lb	3	spray	do.
Acti-dione A (acetate)	5 ppm	4	do.	do.
	20 ppm	4	do.	do.
	50 ppm	4	do.	do.
Acti-dione S (semicarbazone)	5 ppm	4	do.	do.
	20 ppm	4	do.	do.
	50 ppm	4	do.	do.
Acti-dione 0 (oxime)	5 ppm	4	do.	do.
	20 ppm	4	do.	do.
	50 ppm	4	do.	do.
Acti-dione M	50 ppm	5	do.	do.
Chloranil (Spergon SL)	2 lb	5	do.	do.
Copper A[a]	4 lb	3	do.	do.[b]
Folpet(Phaltan)-Copper combination[a]	4 lb	3	do.	do.[b]
Nickel sulfate[a]	100 ppm	3	do.	do.[c]

[a] Tested in 1960, others in 1957.

[b] Resulted in significant increase in incidence of mildew on primary spears.

[c] Phytotoxic.

[d] Soil application made in effort to destroy oospores.

[e] Mylone = 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione.

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[2] Formerly Research Assistant, Oregon State University, now Plant Pathologist, Campbell Soup Company, Ltd., Brampton, Ontario, Canada.

[3] Natti, J. J., G. E. R. Hervey, and C. B. Sayre. 1956. Factors contributing to the increase of downy mildew of broccoli in New York State and its control with fungicides and Agrimycin. Plant Disease Reptr. 40: 118-124.

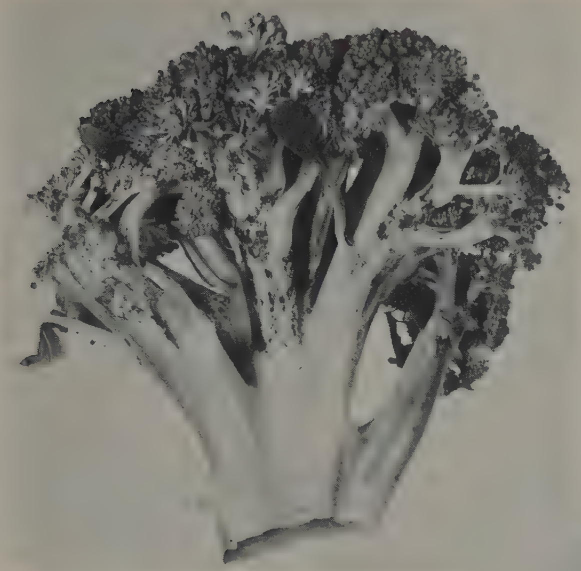

FIGURE 1. (left) Severe discoloration of a broccoli head infected with *Peronospora parasitica* Fr.

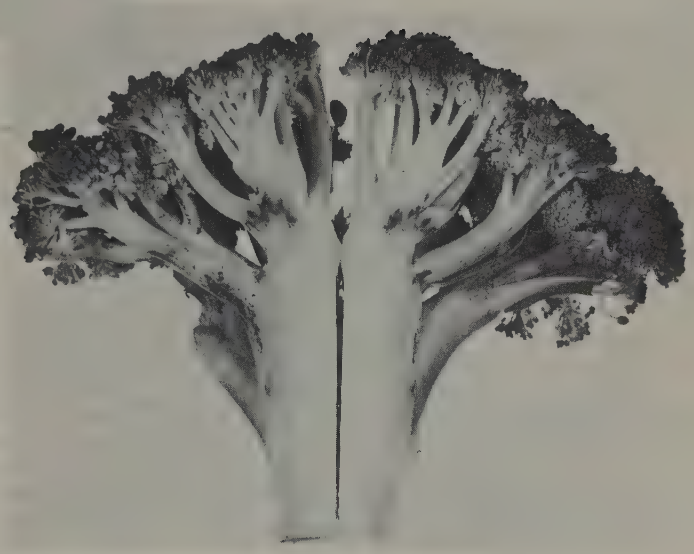

FIGURE 2. (right) Longitudinal section of a diseased head showing the internal discoloration.

DISCUSSION

The chemicals tested in these experiments did not control the disease. Several factors probably contributed to the failure: 1) Under the conditions of the tests the chemicals were not sufficiently fungicidal to *Peronospora parasitica*. 2) It is difficult to distribute the spray or dust evenly on the surface of the heads. 3) The broccoli "head" develops very rapidly and fungicide applications at very frequent intervals are necessary to provide adequate protection. Such frequent applications could result in residue problems.

Research on downy mildew has indicated that the internal discoloration of the broccoli tissue is not caused by a systemic infection in the usual sense. No evidence was found that the fungus moves upward through the vascular tissue from leaf or crown infections into the "head" or side shoots. It has been concluded that infection occurs superficially on the center "head" and side shoots, from where it may penetrate 3 to 4 inches into the tissue. Usually the discolored areas are limited in size and depth of penetration. This results in a "peppered" appearance of the surface.

Resistant varieties are being developed and undoubtedly are the best means of control of this disease.

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FUNGI ASSOCIATED WITH GLADIOLUS ROOTS UNDER CONTINUOUS CROPPING CONDITIONS[1]

Curtis R. Jackson[2]

Summary

Curvularia trifolii, Rhizoctonia solani, Penicillium funiculosum and Fusarium spp. were the most frequent and abundant fungi isolated from necrotic gladiolus roots of successive crops growing in soil-filled concrete tiles during a 14-month period. No great differences in root disease estimates or numbers of isolated pathogens were found among soils which had different cropping histories. Possible relationships between rainfall and the abundance of Fusarium spp. and R. solani were found.

Various pathogenic fungi that attack gladiolus roots have been described in the literature. With the exception of Fusarium oxysporum f. gladioli (Massey) Snyd. & Hans. and Stromatinia gladioli (Dray.) Whet., little is known of the extent of gladiolus root parasitism by other fungi. Jackson (4, 5, 6) indicated the occurrence and possible importance of root infection by Curvularia trifolii (Kauff.) Boed. and Penicillium funiculosum Thom but provided little information on the incidence of root infection of plants growing in soil that had not been artificially infested with these pathogens. Creager (2) and Bald (1) reported that Rhizoctonia solani Kuehn infected necks and corms but they did not mention root parasitism.

The death of a few gladiolus roots does not invariably lead to striking foliage symptoms or lower growth rates, especially if great varietal vigor and favorable growth conditions for gladiolus are involved. Perhaps for this reason little attention has been given to the fungi that cause seemingly innocuous infections of gladiolus roots. The present study was begun to determine the kinds of fungi associated with gladiolus roots when plants were cultivated in a soil with previous gladiolus cropping history. For comparison, a soil was chosen that had no previous gladiolus history. Some effects of a commercially employed fumigant on fungi in a third soil, previously used for gladiolus culture, were studied concurrently by similar methods.

An additional purpose of this study was to gain information on the possible differences in seasonal abundance of fungi isolated most frequently from gladiolus roots. This was facilitated by sampling gladiolus roots over a period of 14 months using the method outlined by Gould (3) in his studies of gladiolus pathogens, a procedure well suited for an investigation of root diseases involving periodic sampling of roots in a confined volume of soil. This method, although artificial in some respects, overcomes many undesirable features inherent in greenhouse investigations using small soil volumes.

MATERIALS AND METHODS

Gould's (3) placement of tiles was modified so that only approximately half of each 3-foot section of 2-foot-diameter concrete tile was buried vertically in the soil. The residual soil within each tile was removed before filling with the soils used throughout the experiment. Three soils were chosen from surrounding agricultural areas and were used to fill three, 7-tile series to within 6 inches of the top of each tile. The soils were 1) Broward fine sand taken from the upper 8 inches of a field where four previous crops of gladiolus had grown, designated Cortez (initial pH 5.4, moisture equivalent 3.2%); 2) Leon Imokalee fine sand taken from the upper 10 inches of a bahiagrass pasture with no previous gladiolus cropping history, designated Braden River (initial pH 6.1, moisture equivalent 3.8%); and 3) sandy soil that had been repeatedly composted and fumigated through the years and that previously had supported a crop of gladiolus seedlings, designated Manatee (initial pH 6.3, moisture equivalent 3.8%). Sodium n-methyl dithiocarbamate dihydrate (Vapam) drench was used at the rate of 2 qt/100 ft.^{2}, diluted with water, to fumigate the Manatee soil in the tiles.

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[1] Approved by the Director of the Georgia Coastal Plain Experiment Station as Journal Series paper No. 96. This report is based on investigations completed at the Gulf Coast Experiment Station, Bradenton, Florida under project 925.

I wish to acknowledge the valuable assistance of Charlotte G. Stanton, laboratory assistant.

[2] Associate Plant Pathologist, Georgia Coastal Plain Experiment Station, Tifton.

The filled tiles were subdivided into quadrants and reference marks were painted on the lip of each tile. In December 1959, one-quarter of each tile was planted with four Florida Pink variety (size No. 5) gladiolus corms. In January 1960, 28 days later, a second quadrant was planted similarly and one plant with attached corm and roots was dug from the December planting. A third quadrant was planted in February, at which time one plant was dug from each of the December and January plantings. This process was repeated every 28 days until March 1961 and involved 15 harvest periods. Throughout the experiment, corms were husked, selected for freedom from blemishes, washed in tap water, and dried before planting. Plants were watered and fertilized sufficiently to promote vigorous growth. Harvest of plants of the four ages, 28, 56, 84 and 112 days, was possible at the fifth planting period. Plants of four ages were included in each root sampling to overcome any possible differences in susceptibility due to plant age.

The largely intact root systems of all harvested plants were washed and each root system was rated for root abundance and extent of necrosis. The product of root abundance, rated from 2-abundant to 4-sparse, and root necrosis, rated from 1-no necrosis to 6-all roots necrotic, was used as the root disease estimate. Five necrotic or discolored, fibrous or adventitious roots from each plant were surface disinfested in 10% NaOC1 for 3 minutes, placed on potato-dextrose agar (PDA), and incubated at 24°C for 10 days. Discrete fungal colonies growing from roots were identified to genera and enumerated. The species of the most frequent and abundant genera were determined.

RESULTS

During the 14-month sampling period 23 genera of fungi were isolated from surface-disinfested gladiolus roots. Nineteen of the genera were common to the three soils used in the survey. The four species and two genera listed in Table 1 were the most frequent and abundant fungi found. Species of the genera Mucor and Aspergillus were less frequent but moderately abundant during certain periods. The distribution of these less frequent genera was about equal among the three soils employed.

Table 1. Percentage abundance of some fungi isolated from roots of gladiolus growing in three soils with different cropping histories[a].

Fungus	Previous gladiolus crops (Cortez)	Previous gladiolus crops, fumigated (Manatee)	No previous gladiolus crops (Braden River)
Curvularia trifolii	13.4	23.9	15.5
Rhizoctonia solani	10.7	8.4	2.7
Trichoderma viride	0.0	1.0	30.4
Penicillium funiculosum	20.1	19.6	15.2
Penicillium spp. (other)	18.8	18.1	10.9
Fusarium spp.[b]	29.1	21.5	15.5

[a] Calculated by soils as the numbers of the genus or species isolated divided by the numbers of all fungi isolated. For each soil the enumeration of fungi was based on isolations from approximately 1920 root segments obtained over a 14-month period.

[b] The most abundant Fusarium species were F. oxysporum, F. solani, and F. roseum.

Single pathogens were occasionally the only fungi isolated from individual root pieces. Mixtures of several parasites or of parasites and saprophytes were most often isolated from root pieces, and for this reason no good estimate could be made of the relative pathogenicity of the fungi isolated. Growth of plants was excellent for the first 8 months, but gradually became poorer and the experiment was terminated after 14 months because more than 25% of the plants died before harvest. Root disease estimates indicated that although there were initial differences in soil cropping histories and fumigant treatment root necrosis and death were similar for all soils. Mean root disease estimates for the 384 plants harvested from each soil during the 14-month period were 1) Cortez - 7.9, 2) Braden River - 9.6, and 3) Manatee - 9.9. Seasonal or time variations of isolation of three of the most frequent and abundant fungi are shown in Figures 1 and 2 with an indication of the seasonal air temperature means, rainfall, and root disease estimates for the experimental period. Air temperature records gave a good indication of the soil temperatures within the tiles since the surface of the soil in the tiles was about 1 foot above the surrounding ground level. The ordinates of the distribution graphs of fungi isolated from gladiolus roots (Figs. 1 B, 1 C and 2 A) are scaled in units of the numbers of colonies iso-

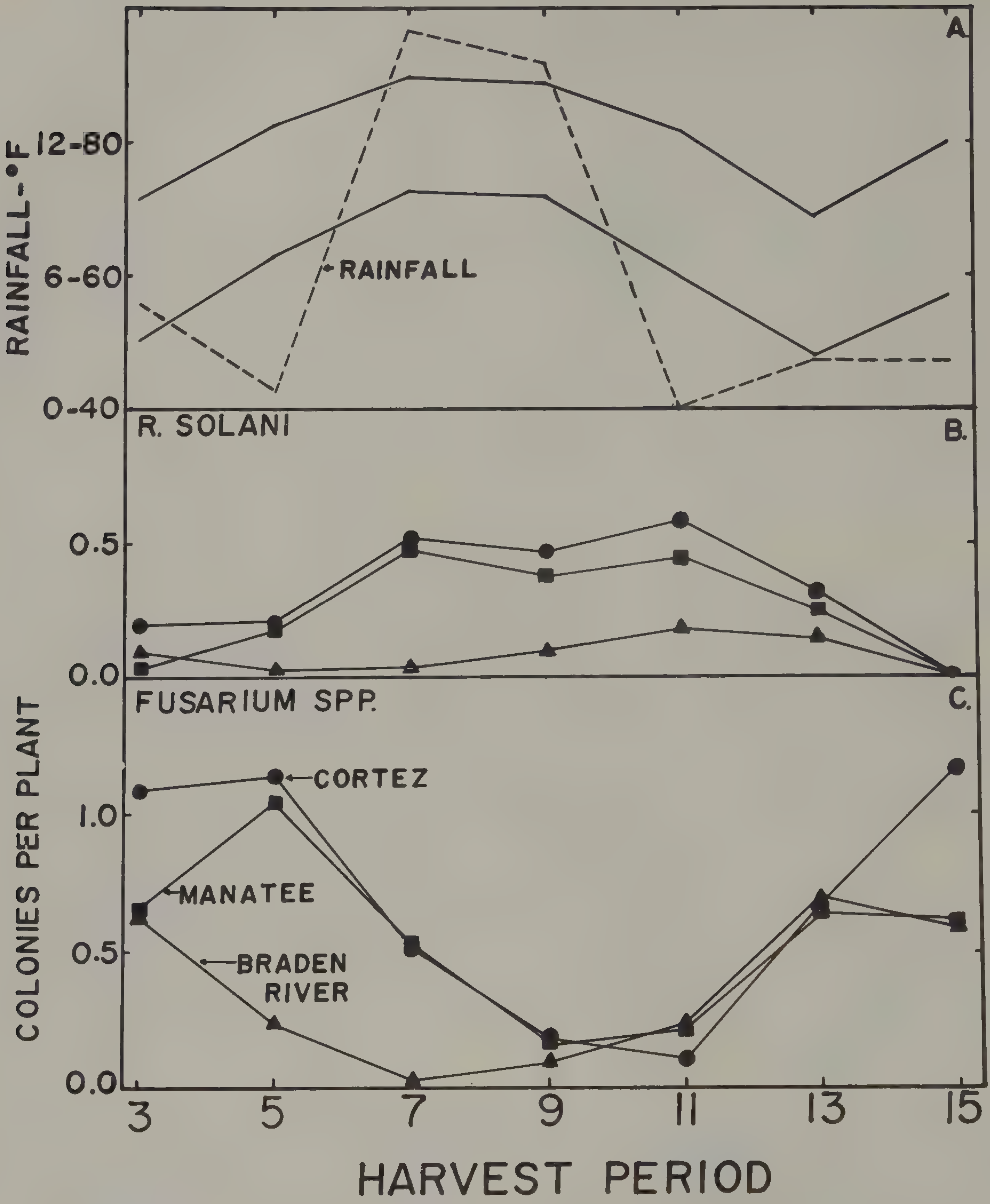

FIGURE 1. A -- Inches rainfall and maximum and minimum mean temperatures for each month corresponding to the indicated harvest period. Colonies per plant of B -- Rhizoctonia solani and C -- Fusarium spp. isolated from gladiolus roots which grew in three different soils are mean figures for the cumulative isolations from two harvest periods. Symbols are explained in Figure 1C.

lated per plant. The plotted points are the average isolation figures from 56 plants (280 roots) for the previous 56-day period (initial harvest period 3 was March 1960 and includes 84 days). Thus a mean figure of 1.0 can be interpreted to indicate that the fungus was isolated once from each plant (one root of the five roots sampled from each plant). Root disease estimates for the corresponding periods (Fig. 2B) are mean values from the same 56 plants used in obtaining mean isolation figures.

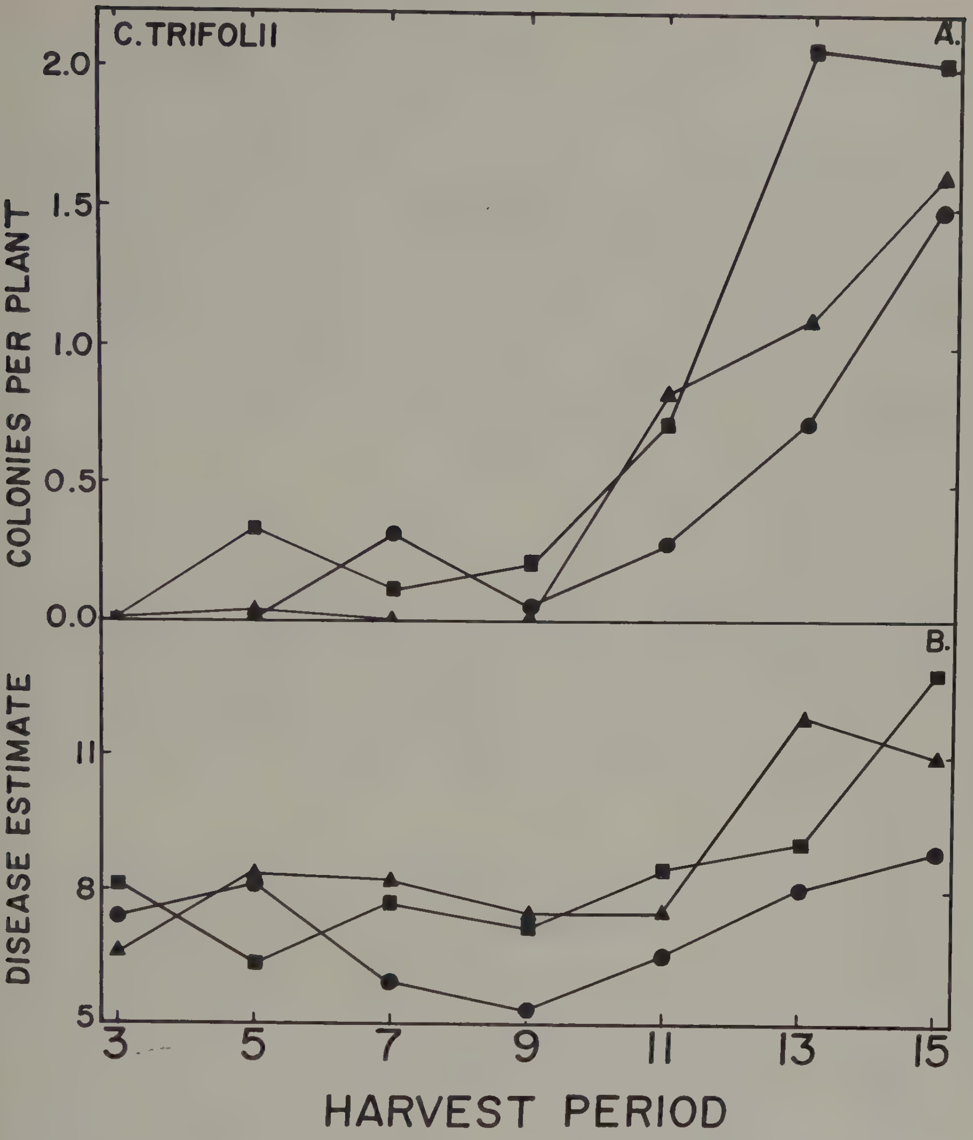

FIGURE 2. A -- Colonies per plant of Curvularia trifolii isolated from gladiolus roots which grew in three different soils are mean numbers of the cumulative isolations of two harvest periods. B -- The disease estimates are mean numbers derived from estimations of the root systems of the same plants from which all isolations were made. Symbols are explained in Figure 1C.

DISCUSSION

The presence of large numbers of at least four recognized gladiolus pathogens among fungi isolated from roots is related most directly to the use of necrotic or discolored roots for isolation material. The relatively great abundance of C. trifolii, R. solani, and P. funiculosum suggests that these fungi may be equal in pathological importance to Fusarium in many cases. R. solani was most abundant in this survey during the summer and fall. Although gladiolus is not cultivated on a commercial scale in Florida during the summer, considerable field infection probably occurs in the fall. Root infection by C. trifolii, as judged by the present results, could limit the production of gladiolus in fields where almost continuous cropping is practiced.

In view of a possible lag between infection of roots of young plants and detection of these infections in 112-day-old plants, a close correspondence between fungal abundance and temperature or rainfall cannot be established. Furthermore, many other factors, such as nematode populations, physical and chemical soil changes, and action of antagonistic organisms, have not been adequately examined in the present study. However, the graphs of isolations of R. solani (Fig. 1B) and Fusarium spp. (Fig. 1C) show definite inter-soil similarity and suggest possible relationships to climatic conditions. A marked decrease in the numbers of isolated Fusarium spp. appears related to an increase of soil moisture since it seems unlikely that the temperatures during the period of this decrease would limit these fungi directly. The abundance of R. solani during the summer and fall may well be related to high temperature and rainfall.

The distribution of P. funiculosum did not appear to be related to climatic conditions or other fungi considered in this study. C. trifolii (Fig. 2A), although not abundant during the first 9 months, increased greatly and was responsible for the premature termination of the experiment. The increase in incidence of C. trifolii infections was not closely associated with temperatures since the optima for root infection are 75° to 90°F (5). Root infection increased while rainfall decreased but this occurrence is possibly more closely related to an increase of soil inoculum during the experiment than to rainfall.

The root disease estimate plotted for Cortez soil is related in form to the graph for Fusarium spp. isolations from the same soil. The other disease estimate graphs show less relationship with Fusarium spp. but do reflect the increase of C. trifolii starting with harvest period 11.

An interesting result of the present study is the indication that root pathogens were active in all soils during most of the experimental period. The use of Braden River soil, which had no previous gladiolus cropping history, resulted in only slightly lower infection (Figs. 1B, 1C and 2A) and root disease estimates were similar to other soils. The use of a fumigant with Manatee soil, even at a higher rate than commercially employed, had only an ephemeral effect on root-infecting fungi. The tiles provided physical separation of the soils but soil infestation from splashing rain or infested corms possibly occurred and may have resulted in early introduction of some additional pathogens into Braden River and Manatee soil.

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ROLE OF TRICHOMES IN CONTACT TRANSMISSION OF VIRUSES[1]

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Summary

When beans with pubescent leaves and cowpeas with glabrous leaves were interrubbed, infection with the cowpea strain of tobacco mosaic virus was about equal whether bean or cowpea was the donor or indicator host, but was less when cowpea was both donor and indicator host. When the pubescent upper surfaces of cucumber cotyledons and the glabrous lower surfaces of cucumber cotyledons were interrubbed, infection with yellow bud mosaic virus and peach ringspot virus was about equal whether the upper or lower surfaces functioned as the donor or indicator surface. However, interrubbing of pubescent surfaces gave more infection than interrubbing of glabrous surfaces. These findings support the belief that trichomes aid but are not necessary for contact transmission of viruses. They do not resolve whether trichomes are more important on the donor host or indicator host.

INTRODUCTION

Contact transmission by interrubbing leaves of diseased and healthy plants is recognized for tobacco mosaic virus (6), potato virus X (8), potato virus F (8), spindle tuber virus (9), potato virus S (11), wheat streak mosaic virus (13), turnip yellow mosaic virus (4), turnip crinkle virus (4), white clover mosaic (2), peach ringspot virus (14, 19), and probably others.

Leaf hairs were implicated in virus transmission by Clinton (6), Allard (1), Sheffield (12), Yarwood (18, 19), and Kontaxis and Schlegel (7). The importance of leaf hairs has been minimized by Boyle and McKinney (3) and Wildman (15).

The present study was stimulated by the accidental discovery of contact transmission of peach ringspot virus (PRSV) to bean in the greenhouse (17), and to squash in the field (14). It seems clear that under the conditions specified, PRSV spread more readily on bean and squash than it did on cherry (16) and peach (5). This difference is correlated with abundant trichomes on bean and squash leaves, and with their relative scarcity on cherry and peach leaves, although there are many other differences. Specific evidence of contact transmission of this virus in fruit trees is not available. Negative results with tests of arthropods as vectors, and the observation that the disease spreads more frequently to adjacent trees than to more distant ones (16), support the idea of contact transmission.

The idea explored in this study was that if trichomes favored contact transmission, more infection would result from interrubbing of pubescent plant parts than by interrubbing of glabrous plant parts. Further, if trichomes on the donor host favored infection more than trichomes on the indicator host, this would suggest that the trichomes functioned as a means of abrasion as well as a source of virus. If, however, trichomes on the indicator host favored infection more than trichomes on the donor host, this would suggest that trichomes functioned as a site of entry of the viruses.

METHODS

The present studies were made with the cowpea strain of tobacco mosaic virus (CTMV) on bean (Phaseolus vulgaris varieties Pinto, Bountiful and Pencil Pod) and cowpea (Vigna sinensis var. Blackeye), peach ringspot virus (PRSV) on bean and cucumber (Cucumis sativus var. National Pickling), peach yellow bud mosaic virus (YBV) on cucumber, and apple mosaic virus (AMV) on cucumber. Cowpeas, almost glabrous, and beans, highly pubescent, are about equally susceptible to mechanical inoculation with virus suspensions on their pubescent upper surfaces as on their glabrous lower surfaces. These surfaces were used as donor and indicator surfaces for YBV, PRS, and AMV. Systemically infected plants, inoculated 15 to 30 days earlier, usually served as donor hosts.

All plants were grown in pots in the greenhouse. For interrubbing inoculation with CTMV (Table 1), healthy bean or cowpea plants 9 to 15 days from seeding were drawn across donor plants so the leaves would rub against each other to simulate wind in a field planting. Heavy

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nitrogen fertilization of the cowpeas was used to enhance early expression of systemic symptoms of CTMV infection. Inoculated plants were well spaced from each other on a greenhouse bench and systemic infections were recorded 10 to 20 days after inoculation.

In controlled surface-to-surface inoculations (Table 2), the upper or lower surface of detached infected leaves were rubbed against the upper or lower surfaces of attached cucumber cotyledons. Infection was later recorded as local lesions or systemic symptoms.

RESULTS AND DISCUSSION

The results of eight trials to test the relation of trichomes to contact transmission of CTMV are given in Table 1. Uninoculated controls never became infected. The high level of infection (44%) that resulted when relatively glabrous cowpeas were interrubbed indicates that trichomes are not necessary for contact transmission. However, much higher infection resulted when one host was pubescent (bean-to-bean, bean-to-cowpea, and cowpea-to-bean). The difference between cowpea-to-cowpea and the other combinations is regarded as highly significant. The fractional transmission (number of plants infected over number of plants inoculated) for inoculation from cowpea to bean was 14/14, 2/2, 4/4, 5/7, 3/4, 4/4, 8/10, and 7/8, whereas the fractional transmission for inoculations from cowpea to cowpea in the same trials was 8/10, 2/2, 2/4, 1/2, 1/4, 0/4, 2/9, and 3/8.

Table 1. Interrubbing transmission of cowpea TMV between bean and cowpea.

Donor host	Indicator host	No. of plants inoculated	% plants infected
Bean (pubescent)	bean	45	90
Bean	cowpea (glabrous)	38	81
Cowpea	bean	53	89
Cowpea	cowpea	43	44

Table 2. Transmission of fruit tree viruses by rubbing infected leaves on cucumber cotyledons.

		Method of interrubbing and total no. of lesions			
Virus	Donor host	Upper to upper	Upper to lower	Lower to upper	Lower to lower
YBV	Cucumber cotyledons[a] (3 trials)	20	5	3	5
YBV	Cowpea leaves[b] (1 trial)	12	1	-	-
PRSV	Cucumber cotyledons (2 trials)	79	17	34	16
AMV	Cucumber true leaves[c] (2 trials)	15	8	16	14

[a] Pubescent on upper surface only.
[b] Glabrous on both surfaces.
[c] Pubescent on both surfaces.

The relative importance of trichomes on donor as compared with indicator host is impossible to determine from these data. The high and approximately equal infection from bean-to-bean and bean-to-cowpea inoculations would indicate that trichomes on the indicator host are of little importance. The high and approximately equal infection from bean-to-bean and cowpea-to-bean inoculations would indicate that trichomes on the donor host are of little importance. The greater infection from bean-to-cowpea than from cowpea-to-cowpea inoculations would indicate that trichomes on the donor host favor infection. The greater infection from cowpea-to-bean than from cowpea-to-cowpea would indicate that trichomes on the indicator host favor infection. The only safe conclusion is that trichomes on either donor or indicator host favor infection.

Results of eight trials of transmission of fruit tree viruses by interrubbing of leaves are given in Table 2. Here also, it is clear that trichomes are not necessary for contact transmission, but their presence favors it. Trichomes on either donor or indicator hosts resulted in more infection than trichomes on only one host, but it is not clear whether trichomes are more important on donor or indicator host.

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BOTRYTIS INFLORESCENCE BLIGHT ON AMERICAN HOLLY IN NEW JERSEY

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A previously undescribed disease affecting the inflorescence of *Ilex opaca* var. Tom Brown and var. Slim Jim was first noted in the Rutgers holly plantings in New Brunswick in June 1960. The blighting of the flower clusters, peduncles, and small twigs of these male cultivars was noted in mid-June during and after a wet and humid period. Water-soaked tan-colored lesions occurred on floral parts and peduncles. The entire flower and flower cluster blighted. This was followed by dieback of the branches supporting the inflorescences (Fig. 1) as well as some of the adjacent previous year's wood. Although proof of pathogenicity was not attempted, all evidence indicates that *Botrytis* sp. is the primary pathogen.

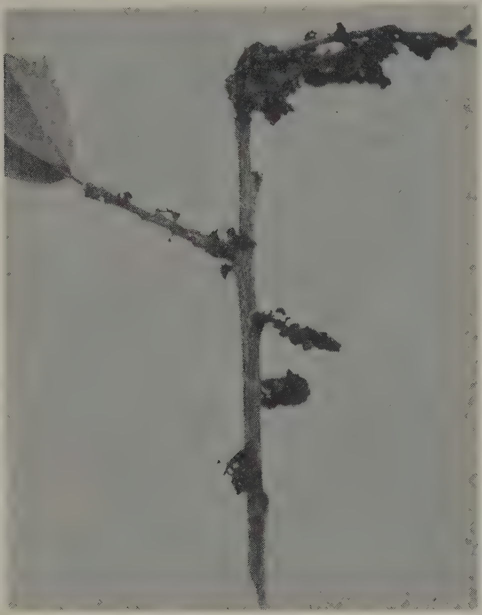

FIGURE 1. Botrytis inflorescence blight and dieback of *Ilex opaca* var. Tom Brown.

Most of the flower clusters of these two varieties were infected, whereas no Botrytis inflorescence blight was noted on the many other American and English hollies in the area. It is believed that the heavy pollen residue, aborted pistils, and old withering flowers may serve as loci of infection. These varieties of American holly produce very heavy bloom and abundant pollen; the Tom Brown variety has been frequently used as a parent in holly hybridization in New Jersey.

Rainfall was recorded in New Brunswick in June 1960 as follows (inches): June 4th, 0.17; 5th, 0.05; 12th, 0.16; 14th, 0.05; 15th, 0.07; 16th, 0.30; 18th, 0.32; 24th, 0.05. Although this is not heavy rainfall for June, from June 12 to 18 rain was sufficiently frequent to keep plants wet for extended periods.

In the spring of 1961, two large Tom Brown holly plants were placed in a cool greenhouse (evaporation-cooling) under conditions of high humidity, in order to extend the pollen-producing period. Very severe infection of the inflorescence developed during the blooming period, followed by very severe dieback of much of the new growth.

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NEW HOSTS FOR THREE HELMINTHOSPORIUM SPECIES FROM CORN

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Abstract

The three Helminthosporium spp. that cause leaf spotting and leaf blights on corn were tested in the greenhouse on 43 gramineous plants representing 12 genera and 14 species. H. turcicum infected 22, of which Coix lacryma-jobi, Tripsacum dactyloides, Setaria italica, and Pennisetum typhoides are new hosts. H. maydis infected 31, with C. lacryma-jobi, Euchlaena mexicana, P. typhoides, Sorghum vulgare (six varieties), T. dactyloides, Avena sativa, Secale cereale, and Triticum aestivum reported as new hosts. H. carbonum (race 1) infected 15, of which C. lacryma-jobi, Setaria italica, Sorghum halepense, and five varieties of S. vulgare are new reports. Race 2 of H. carbonum infected for the first time the same 15 as race 1 and in addition Avena sativa and Secale cereale. The three fungus species have some hosts in common but the patterns of their host ranges are not identical.

Three species of Helminthosporium commonly attack Zea mays (corn). H. turcicum Pass. causes northern leaf blight, H. maydis Nisik. & Miyake, southern leaf blight, and H. carbonum Ullstrup, leaf spot. Little is known of the extent of the host range of these three important species of Helminthosporium.

Besides corn, H. turcicum is generally reported as occurring naturally on various varieties of Sorghum vulgare (broomcorn, sorghum, sorgo, and kafir), S. halepense (Johnsongrass), and S. sudanense (Sudangrass). H. turcicum has been found also on leaves of Panicum miliaceum from New South Wales (4), P. novemnerve from Southern Rhodesia (10), Paspalum conjugatum from the Gold Coast (11), Sorghum margaritiferum from Sierra Leone (3), and Euchlaena mexicana from Honduras (16), Mexico (23), and Texas (22). Before 1900, Atkinson (1) reported H. turcicum from Alabama on leaves of Cinna arundinacea (stout woodreed) and on Elymus sp. Shoemaker (20), who studied the Atkinson collections of Helminthosporium spp. from Alabama, concluded that H. turcicum on Elymus sp. of the early record would now be identified as H. sorokinianum Sacc., and on C. arundinacea would be identified as H. leersii Atk. Other workers (2, 5) also have questioned the occurrence of H. turcicum on C. arundinacea and Elymus spp. Ghimpu (9) claimed that H. turcicum affected tobacco seedlings in beds. Preliminary tests by the author failed to produce any symptoms on tobacco seedlings.

Helminthosporium maydis is most commonly known on corn but is reported also on other hosts which include Sorghum halepense (8) and S. guineense (19), (Guinea corn). In 1936, Galloway (7) isolated a fungus from Euchlaena mexicana which he thought to be H. maydis. Drechsler (6) previously had mentioned the possibility of H. maydis on some herbarium specimens of E. mexicana. No proof was obtained in either of these instances. Kovachich (12), in 1957, associated conidia of H. maydis with rotted leaves of Elaeis guineensis (oil palm) and with the rotting of Sarcophrynium leaves in the Belgian Congo. His line drawings would fit conidia of this species of Helminthosporium but there was no positive proof through isolation and reinoculation that the fungus was H. maydis. Later, Tarr (21) listed the disease on E. guineensis in the Sudan as being caused by H. stilbaceum (Moreau) Hughes. A cultural study of a fungus isolated from Zingiber officinale (ginger) from India showed it to be a strain of H. maydis (14).

Kovachich (12) also described on Elaeis guineensis a disease caused by Helminthosporium carbonum, which he called "eye spot." The fungus was isolated from leaf lesions and inoculated on E. guineensis to produce the "eye spot." H. carbonum was also found sporulating on Sarcophrynium arnoldianum, a common weed in oil palm nurseries. Molot (15) tested a new Helminthosporium from corn in France and found that it infected sorghum. The fungus is similar to H. carbonum but he thought it was different from the two strains known in the United States. He pointed out that in 1957 C. M. Messaien had isolated a strain of H. carbonum at Saint-Martin-de-Hinx in France that was very similar to H. carbonum race 2 but different from the one Molot isolated in 1958. Both strains from France produced lesions on corn and sorghum leaves. Generally H. carbonum is known to attack corn. Race 1 of the

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Table 1. Infection reactions of plants from various species of the Gramineae to inoculation in the greenhouse with three *Helminthosporium* species from corn.

Species number	Species inoculated	Reactions[a] to indicated fungus			
		H. turcicum	*H. maydis*	*H. carbonum* 1	*H. carbonum* 2
3	*Coix lacryma-jobi*	+	+	-	-
4	*C. lacryma-jobi*	-	+	-	-
5	*C. lacryma-jobi*	-	--	-	-
6	*C. lacryma-jobi*	-	-	-	-
8	*C. lacryma-jobi*	+	+	+	+
41	*C. lacryma-jobi*	-	--	--	--
9	*Euchlaena mexicana*	+	+	+	+
16	*E. mexicana*	+	+	+	+
63	*E. mexicana*	+	+	+	+
64	*Pennisetum typhoides*	+	+	-	-
13	*Setaria italica*	+	-	+	+
65	*S. italica*	+	-	+	+
15	*S. glauca*	-	-	-	-
14	*Sorghum halepense*	+	-	+	+
	S. vulgare				
37	Rancher	-	+	-	-
58	Honey	-	+	+	+
59	Wiley	-	-	-	-
60	Sart	-	-	-	-
61	Black Amber	-	+	+	+
54	Red leaf	-	+	-	-
55	Sooner	-	+	+	+
56	Hybrid 610	-	+	+	+
57	Combine	-	+	+	+
66	*S. sudanense*	+	+	+	+
110	*Tripsacum dactyloides*	+	+	-	-
	Zea mays				
62	Gatoni (pop corn)	+	+	-	-
53	PJMC (sweet corn)	+	--	+	+
00	L317	+	+	+	+
12	AFRO 53297/350	+	+	-	+
33	AFRO 474	+	+	-	+
18	Kys	--	+	-	--
19	CI. 21	+	+	-	+
80	CI. 64	+	+	-	+
81	K64	+	+	-	+
83	CI. 90A	+	+	-	--
98	P. I. 198902 (Argentina)	+	+	-	+
101	P. I. 186208 (South Africa)	+	+	-	+
	Secale cereale	--	+	-	+
	Avena sativa				
	Clinton	-	+	-	+
	Boone	-	+	-	+
	Triticum aestivum	-	+	-	-
	Hordeum vulgare	-	-	-	-
	Oryza sativa	-	-	-	-

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[a] + = infection without regard to degree; - = no infection, and -- = no inoculation made.

fungus is selective as to the strains of corn it attacks and race 2 is more general.

Mitra (13) observed that *H. turcicum* caused limited leaf lesions on *Avena sativa*, *Hordeum vulgare*, *Oryza sativa*, and *Saccharum officinarum*, and a somewhat heavier infection on *Triticum aestivum* when young plants were inoculated in the greenhouse. Nisikado and Miyake (17) reported that *H. maydis* caused definite lesions on *Setaria glauca*. According to Robles (18), *H. carbonum* infected *Euchlaena mexicana* in greenhouse and field inoculations.

As far as is known by the author no hosts except those mentioned are reported[2] as being infected by *H. turcicum*, *H. maydis*, or *H. carbonum*.

MATERIALS AND METHODS

At Plant Industry Station, Beltsville, Maryland, the reactions of 43 different gramineous plants were tested in the greenhouse with *Helminthosporium turcicum*, *H. maydis*, and *H. carbonum* (races 1 and 2) isolated from corn. Inoculations were made separately when the plants were about 1 month old. Composite inocula of each of the fungi were prepared by homogenizing agar plates of five isolates in water and diluting so that the ratio was 1 plate of culture to 1000 cc of water. To inoculate, plants in two 4-inch pots (four plants/pot) were sprayed with the composite inoculum; one pot containing four plants served as a check. Inoculated and check plants were placed for 36 hours at 25° C in inoculation chambers equipped with intermittent sprayers to maintain high levels of moisture. They were then removed from the moist chambers and placed on a greenhouse bench to allow disease to develop. In each instance of infection the fungus used for inoculation was reisolated.

RESULTS AND CONCLUSIONS

Table 1 gives the data on infection without indicating the severity. New hosts that *H. turcicum* was found to infect were two of the six selections of *Coix lacryma-jobi* (Job's tears), *Setaria italica* (German foxtail millet), *Pennisetum typhoides* (Starr pearl millet), and *Tripsacum dactyloides* (Eastern gamagrass). Lesions were inconspicuous on *C. lacryma-jobi*, small on *T. dactyloides* and *P. typhoides* but were more conspicuous and with more necrosis on *S. italica*.

H. maydis is recorded as infecting for the first time three selections of *Coix lacryma-jobi*, the three selections of *Euchlaena mexicana* tested, *Pennisetum typhoides*, six of the *Sorghum vulgare* varieties, *Avena sativa*, *Secale cereale*, *Triticum aestivum*, and *Tripsacum dactyloides*. The three selections of *E. mexicana* were heavily infected; the other hosts attacked for the first time developed light to moderate infections.

H. carbonum (races 1 and 2) is noted as infecting for the first time one of the selections of *Coix lacryma-jobi*, the two selections of *Setaria italica*, *Sorghum halepense*, five of the *S. vulgare* varieties, *S. sudanense*, a PJMC sweet corn variety, and one inbred line of corn, L317. Race 2 of *H. carbonum* also infected rye and oats. The infections were heavy on *C. lacryma-jobi*, *S. sudanense*, and *Setaria italica*, and were light to moderate on the other hosts.

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[2] Since the conclusion of these experiments and the completion of this manuscript, results of another study have been published (Nelson, R. R., and D. M. Kline. 1961. Plant Disease Reptr. 45: 644-648) in which the three corn *Helminthosporium* spp. are reported to attack other hosts when inoculated in the greenhouse. Only one of these is given herein as a new report in this study: *H. carbonum* on *Sorghum sudanense*.

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SYMPTOM STABILITY IN SWEET CHERRY TREES AFFECTED BY CRINKLE LEAF AND DEEP SUTURE[1]

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Summary

Thirteen sweet cherry orchards, totaling 2669 trees, were mapped and surveyed on a tree-by-tree basis from 1952 through 1957. Presence of crinkle and/or deep-suture symptoms in each tree was recorded annually in July.

The conclusions were reached that 1) incidence of crinkle and deep-suture symptoms varies from year to year in affected orchards, 2) this is particularly evident in orchards where most of the affected trees exhibit only mild symptoms, 3) symptoms can be expected every year only in those trees that are affected by severe forms of the two disorders, and 4) the two disorders may precede each other or occur together.

INTRODUCTION

Growers of sweet cherries in Idaho have, on certain years, contended that the so-called genetic condition known as crinkle leaf (1) spreads in their orchards. This has suggested annual fluctuations in symptom expression, either in quantitative incidence or in severity of symptoms in individual trees.

A preliminary survey showed that both crinkle and deep suture (2) were more common in some orchards than in others. No affected trees were found in some orchards; many were found in others. This was particularly true for crinkle. Severity and distribution of symptoms in individual trees also was observed to vary greatly.

In order to reveal whether significant annual fluctuation occurred in incidence of symptoms of both disorders, orchards were selected for tree-by-tree examination over a period of years.

MATERIALS AND METHODS

Thirteen orchards were selected which contained 2669 trees ranging in age from 3 to approximately 40 years, that is, orchards in which there had been intermittent or continuous replanting over a prolonged period. This was done in order to observe old and young trees in the same environment. The selected orchards were chosen from among orchards totaling 5697 trees, which were examined in the preliminary survey.

Beginning in 1952, symptoms of crinkle and deep suture were recorded on orchard maps in mid-July. Trees were examined on all sides at each inspection. Symptoms were recorded as either present or absent, because the scope of the study prohibited individual tree mapping; and, therefore, prohibited recording detailed data on severity of symptoms in the various parts of individual trees.

Since suture depressions of uncertain cause sometimes occur in sweet cherry fruits, deep-suture symptoms were recorded for a tree only if both leaf and fruit symptoms were present. Crinkle symptoms were recorded only when affected leaves showed both the irregular margins and the fine-line chloroses associated with the condition.

RESULTS

Development of crinkle symptoms in individual trees did not appear to be influenced by tree age. Recently planted trees were affected as well as older trees. However, deep-suture symptoms seldom were found on trees less than about 15 years of age.

Crinkle symptoms were much more prevalent than symptoms of deep suture (Fig. 1).

During the 6 years of detailed tree survey, there was wide fluctuation in incidence of both disorders (Fig. 1). Symptoms were especially prevalent in 1953. Fluctuations in incidence of the two disorders paralleled each other in striking fashion, as Figure 1 indicates.

Recurrence of symptoms in the same tree for prolonged periods was much less common (compare Figs. 1 and 2R) than expected; and, when the duration of recurrence in individual

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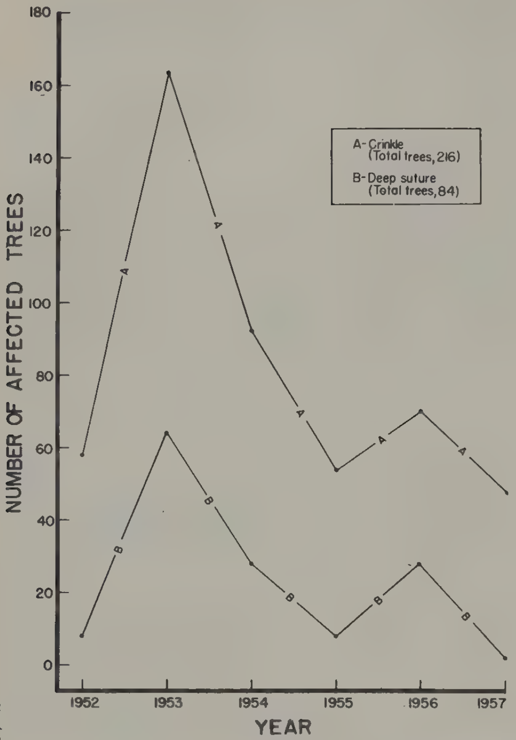
[1] Approved by the Director as Research Paper No. 540; study conducted as a part of Western Region Project W-64.

[2] Associate Plant Pathologist, University of Idaho Agricultural Experiment Station.



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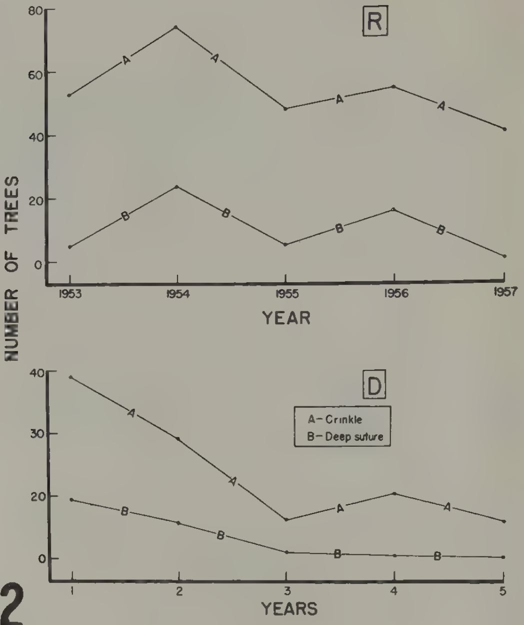

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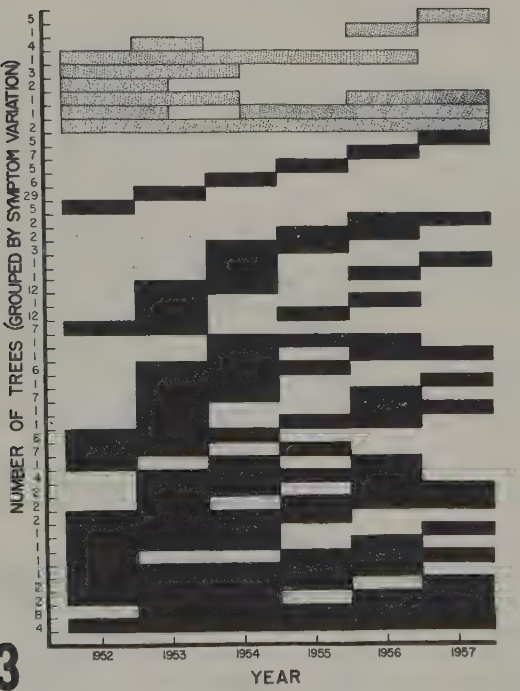

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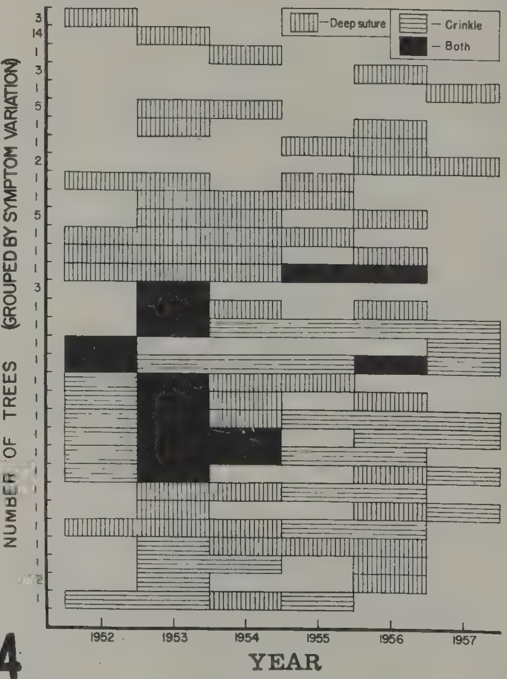

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FIGURE 1. Annual incidence of crinkle and deep-suture symptoms in 13 sweet cherry orchards totaling 2669 trees.

FIGURE 2. Recurrence (R) of crinkle and deep-suture symptoms in previously affected sweet cherry trees in 13 orchards totaling 2669 trees, and duration (D) of the recurrence in individual trees.

FIGURE 3. Annual variation in symptom expression in crinkle-affected trees in selected Bing (black bars) cherry orchards with black Tartarian (stippled bars) polenizers.

FIGURE 4. Annual variation in symptom expression in selected Bing cherry trees affected by deep suture and by both crinkle and deep suture.

trees was studied (Fig. 2D), few trees were found to be permanently symptomatic. Observations indicated that the trees that retained their symptoms over the longest periods were those that had the most severe symptoms.

Most of the severe cases of crinkle occurred in trees of the Black Tartarian (Prunus avium) variety, although equally severe cases occasionally were found in the Bing (P. avium) variety (Fig. 3).

Recurrence of symptoms of both crinkle and deep suture generally was not continuous during the 6-year period of the study (Figs. 1, 2). Interruptions were commonplace, with only the most severe forms continuing to show in the same trees year after year.

Several trees were affected by both crinkle and deep suture (Fig. 4). Sometimes one condition preceded the other, and occasionally both were present in the same tree at the same time.

DISCUSSION AND CONCLUSIONS

Since the crinkle and deep-suture conditions are not reproducible in inoculated trees with presently known methods, all observations in a study of this kind must be evaluated without the benefit of graft-transmission data. Bud-grafting studies (not reported here) yielded only information concerning bud-perpetuation of the symptoms. Such studies were unsatisfactory, both because donor-bud take was poor and because donor-bud growth on healthy trees often was unaffected, even when the buds were taken from relatively severe cases in the surveyed orchards.

Therefore, since symptom evaluations in this study had to be entirely visual, the possibility exists that conditions other than those described as crinkle (1) and deep suture (2) may have influenced observations. However, after exhaustive study of the symptoms found in the surveyed orchards, before, during and after the study herein reported, the writer believes that the only significant differences among symptoms of each disorder were those of varying degrees of severity; that is, on one extreme were the strong and annually recurring symptoms and on the other the mild and transient symptoms.

The study revealed 1) that severe forms of both disorders tend to recur continuously in affected trees year after year (as reported by others); 2) that milder forms may be present one year but not another; 3) that total number of affected trees varies considerably with different years, particularly where most of the trees are affected by mild forms; 4) that either disorder may precede the other in affected trees; and 5) that the two conditions may occur together.

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CONTROL OF PLANT-PARASITIC NEMATODES AND WEEDS IN HOLLY NURSERIES

Frank A. Haasis and J. N. Sasser

Summary

All-purpose soil fumigants (MC-2, Brozone, and Trizone) controlled plant-parasitic nematodes and weeds sufficiently to allow field establishment of transplanted liners of two cultivars of *Ilex crenata* and to permit continued excellent growth. Plants grown in fumigated sites were of superior quality as measured by compactness, uniformity and vigor, were more winter hardy, and required one third less time to attain salable size than the controls.

Damage by plant-parasitic nematodes in the production and maintenance of woody ornamentals is widely recognized (3, 4, 7). The increasing demand for nematode-free stock presents a difficult problem for the producer of woody ornamentals. Control of plant-parasitic nematodes and other biotic competitors has been studied for many economic crops and more recently certain of these methods have been studied relative to the cultivation of nursery stock (2, 5). These studies were designed to determine 1) the effects of soil fumigants on the control of plant-parasitic nematodes and weeds; 2) the reinfestation of weeds; 3) subsequent population buildup of nematodes; and 4) the effect of treatment on growth and development of two cultivars (*Rotundifolia* and *Convexa*) of *Ilex crenata*.

METHODS

Two formulations of methyl bromide (MC-2 and Brozone)[1] and a mixture containing methyl bromide, chloropicrin and propargyl bromide (Trizone)[2] (8), were compared with a non-fumigated control in a nursery site classified as Norfolk sand. The fumigants were applied in mid-March when soil moisture was near optimum for soil fumigation and soil temperature (6-inch depth) was 48° to 52° F. Methyl bromide (MC-2) was vaporized with heat (1) and Brozone was pressure injected (30 psi), each at 217 and 434 lb/A. Trizone was injected by means of a gravity flow applicator at rates of 140, 200, and 260 lb/A. All injections were made on 10-inch centers at a depth of 5 inches. The fumigants were applied simultaneously with the laying and sealing of 2-mil polyethylene covers with tractor-mounted equipment (Fig. 1) of special design (8). Covers were removed manually after 48 hours. In order to minimize contamination, alleyways (Fig. 1), approximately 20 inches wide, between plots were treated manually with methyl bromide (434 lb/A). Well developed liners of two cvs. of *Ilex crenata* that had been propagated in sterilized media were planted (split block design) in plots 50 x 5 feet. Liners were planted on 2.5 x 3 foot centers 5 weeks after treatment. Standard procedures involving fertilization, irrigation, mulching, spraying, and pruning were used. The plots were continued for 30 months.

Weed populations were recorded 2 and 3 months after treatment. A modified Baermann funnel technique (6) was used to estimate nematode populations prior to treatment and at varying intervals after fumigation throughout the duration of the test. Soil samples consisted of core profiles taken within the root zone of plants at random stations in each plot or deliberate collections from the rhizosphere of plants taken at random in each plot. Plant response to treatment was evaluated by size (crown diameter), compactness, and tolerance to cold injury.

RESULTS

Nematode Control: Pretreatment assays revealed a uniform but low population (av. 50/pint of soil) of the spiral nematode *Helicotylenchus nannus* Steiner and scattered traces of the pin nematode *Paratylenchus* sp.[3].

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[1] MC-2 = methyl bromide +2% chloropicrin; Brozone = methyl bromide in an organic solvent (Dow Chemical Co.).

[2] Trizone = (Dow Chemical Co.)

[3] The species of *Paratylenchus* encountered in this study is morphologically similar to *P. microdorus* Andrassy which is described from females only. The North Carolina form differs from the type by the presence of males which exhibit distinct sexual dimorphism and by having larger females (322-406μ vs. 388-420μ) - By courtesy of Dr. H. Triantaphyllou.

Table 1. A comparison of MC-2, Brozone and Trizone on weed control 8 and 12 weeks after fumigation.

Treatment	Method of application	Dosage lb/A	Av. number of weeds/sq. ft. 8 weeks	12 weeks	Mean
MC-2	Vaporized	217	0.2	1.2	0.7
do.	do.	434	0.1	1.0	0.5
Brozone	Injected	217	0.5	1.4	0.9
do.	do.	434	0.1	0.8	0.4
Trizone	Injected	140	2.4	4.6	3.5
do.	do.	200	0.4	1.6	1.0
do.	do.	260	0.1	1.0	0.5
Check	---	none	31.2	30.3	30.7

Table 2. Effect of soil fumigation with MC-2, Brozone or Trizone on populations of plant-parasitic nematodes associated with cvs. of Ilex crenata. Assays from core profiles of soil.

Treatment	Method of application	Dosage lb/A	Kind of nematode	Number of nematodes recovered per pint of soil: 1959 Sept. 8	1959 Nov. 18	1960 May 16	1960 Sept. 20	1961 April 4
MC-2	Vaporized	217	P[a]	0	0	12	12	42
			S	0	0	0	0	44
do.	do.	434	P	0	0	162	544	44
			S	0	0	0	0	0
Brozone	Injected	217	P	0	0	166	112	69
			S	0	0	0	0	0
do.	do.	434	P	0	0	0	6	0
			S	0	0	0	0	0
Trizone	Injected	140	P	0	0	0	25	31
			S	0	0	0	0	0
do.	do.	200	P	0	0	44	62	94
			S	0	0	0	0	0
do.	do.	260	P	0	0	44	0	125
			S	0	0	0	0	12
Check	----	none	P	50	0	920	262	425
			S	75	33	300	81	125

[a] P = pin nematodes (Paratylenchus sp.). S = spiral nematodes (H. nannus).

Table 3. Effect of soil fumigation with MC-2, Brozone or Trizone on populations of plant-parasitic nematodes associated with cvs. of Ilex crenata. Assays from rhizosphere samples.

Treatment	Method of application	Dosage lb/A	Kind of nematode	Number of nematodes recovered per pint of soil, host and date of sampling in 1961: cv. Rotundifolia Jan. 5	cv. Rotundifolia Aug. 31	cv. Rotundifolia Sept. 12	cv. Convexa Jan. 5	cv. Convexa Aug. 31	cv. Convexa Sept. 12
MC-2	Vaporized	217	P[a]	0	-	125	0	-	0
			R	0	-	0	0	-	0
			S	0	-	0	0	-	0
do.	do.	434	P	12	-	[illegible]31	50	-	0
			R	0	-	0	0	-	0
			S	0	-	0	0	-	0
Brozone	Injected	217	P	0	-	900	2	-	0
			R	0	-	0	0	-	0
			S	0	-	0	0	-	0
do.	do.	434	P	0	-	[illegible]9	0	-	12
			R	0	-	0	0	-	0
			S	0	-	0	31	-	0
Trizone	Injected	140	P	0	-	200	25	-	0
			R	0	-	0	0	-	0
			S	0	-	0	0	-	0
do.	do.	200	P	75	-	381	0	-	19
			R	0	-	0	0	-	0
			S	0	-	0	0	-	0
do.	do.	260	P	6	-	975	0	-	62
			R	0	-	0	0	-	0
			S	0	-	0	0	-	0
Check	----	none	P	109	3537	2975	175	485	1731
			R	0	350	494	0	175	281
			S	817	2312	2094	300	3312	2806

[a] P = pin nematode (Paratylenchus sp.); R = ring nematode (Criconemoides sp.); S = spiral nematode (H. nannus).

Table 4. Response of two cultivars of Ilex crenata to soil fumigation with MC-2, Brozone or Trizone[a].

Treatment	Method of application	Dosage lb/A	Crown diameter 1	Crown diameter 2	Crown diameter Mean	Plant compactness 1	Plant compactness 2	Plant compactness Mean	Cold injury 1	Cold injury 2	Cold injury Mean
				(Inches)			(Index)			(Index)	
MC-2	Vaporized	217	11.7[b]	13.4	12.5	2.4	2.6	2.5	0.22	1.12	0.67
do.	do.	434	12.2	15.1	13.6	2.7	3.2	2.9	0.05	0.40	0.22
Brozone	Injected	217	12.8	14.3	13.5	3.0	3.0	3.0	0.52	0.87	0.69
do.	do.	434	13.2	15.7	14.4	3.3	3.7	3.5	0.10	0.25	0.17
Trizone	Injected	140	12.9	16.0	14.4	2.9	3.7	3.3	0.02	0.15	0.08
do.	do.	200	13.0	16.1	14.5	3.0	3.8	3.4	0.07	0.12	0.09
do.	do.	260	13.5	15.7	14.6	3.3	3.9	3.6	0.05	0.22	0.13
Check	----	none	9.4	11.6	10.5	1.8	1.9	1.8	1.75	3.25	2.50
LSD 0.1					0.671			0.387			

[a] 1 = cv. Rotundifolia; 2 = cv. Convexa.

[b] Values of the cultivars represent the average of 80 plants; mean values are the average of the sum of the two cultivars.

Post treatment assays 4 and 7 months after date of planting failed to detect either spiral or pin nematodes in any fumigated plot, whereas low but uniform populations of both forms were recovered in the control plots at each sampling period. One year after planting, however, pin nematodes were found in moderate numbers in many of the fumigated plots and in substantial numbers in control plots; spiral nematodes, on the other hand, were recovered from control plots only. This trend continued throughout the second year, but during the third year a few spiral nematodes began to appear in two fumigated plots (Table 2).

Population estimates of nematodes based on assays of rhizosphere samples collected 20 and 28 months after planting evidenced the virtual absence of spiral and ring nematodes in fumigated plots irrespective of material or dosage (Table 3). Pin nematodes were recovered in moderate numbers in all fumigated plots containing the cv. Rotundifolia and in significantly less numbers from similar plots containing the cv. Convexa, (Table 3). Substantial numbers of the three genera were recovered in late summer collections from non-fumigated plots; lesser numbers of pin and spiral nematodes and no ring nematodes were recovered from the same control plots 8 months earlier (Table 3).

Weed Control: Goose grass (Galium aparine), rough bed straw (Galium asprellum), crab grass (Digitaria sanguinalis), yellow nut sedge (Cyperus esculentus), annual sedge (Cyperus compressus), spurge (Spergula arvensis), and blue toad flax (Linaria canadensis), occurred most frequently in areas adjacent to the test plots and in the nonfumigated plots with yellow nut sedge, spurge, blue toad flax and crab grass predominating (Fig. 2). All fumigation treatments, except the lowest dosage of Trizone, gave excellent weed control (Table 1). The germination and growth of annual sedge was favored significantly in plots treated with Trizone at a dosage of 140 lb/A whereas all other weeds were effectively controlled.

Plant Response: Each cv. of I. crenata made better and more uniform growth in less time in fumigated plots (Fig. 3) than in nonfumigated plots (Fig. 4) (Table 4). Plants receiving the minimum dosage (217 lb/A) of either formulation of methyl bromide were inferior to those that received 434 lb/A. Excellent plants were produced in all of the plots treated with Trizone. In addition, plants grown in fumigated plots showed less damage from cold injury than those in the nonfumigated plots, although the cv. Rotundifolia exhibited greater natural winter hardiness than the cv. Convexa (Table 4).

The economic gain attributable to the use of all-purpose soil fumigants ranged from 100 to 247% greater for treated versus nontreated plots with cv. Rotundifolia and 15 to 25% greater with cv. Convexa.

DISCUSSION

Under the conditions of these experiments, populations of plant-parasitic nematodes were reduced below detectable levels by soil treatment with all-purpose fumigants. Furthermore, weed populations were reduced sufficiently to permit excellent establishment of transplanted liners of I. crenata without the need of additional control measures.

Populations of Paratylenchus sp. increased to detectable levels after 1 year in several fumigated plots (Table 2), and continued to increase largely on cv. Rotundifolia during succeeding months (Table 3). By contrast, populations of spiral and ring nematodes were not detected in treated plots. The data suggest that pin nematodes increased from a low level residuum which resisted fumigation rather than from sources outside the plots.

Reinfestation by weeds occurred mainly in areas where an adjacent plot was untreated. In these instances, weed intrusion from contiguous plots was largely restricted to yellow nut sedge which invaded by extension of underground stolons. Once invasion was achieved, maturation of tubers followed. Reinfestation by wind dissemination of seed was insignificant.

The advantage of soil treatment with all-purpose fumigants lies in the restoration of problem sites to high level crop production. Some of the important factors involved in this restoration include 1) production of superior quality plants, 2) a 25 to 40% reduction in growing time, 3) production of plants better adapted to withstand climatic stresses, 4) more efficient utilization of fertilizer or other cultural amendments, and 5) production of uniform material (Fig. 5).

The gross monetary gain attributable to soil treatments used ranged from $4800.00 to $6900.00 for cv. Rotundifolia and from $2000.00 to $3600.00 for cv. Convexa, as extrapolated on an acre basis. The cost of treatment, on a commercial scale, approximates $400.00 to $600.00 per acre. This investment in many instances is regained the first season of operation through savings in weed control alone.

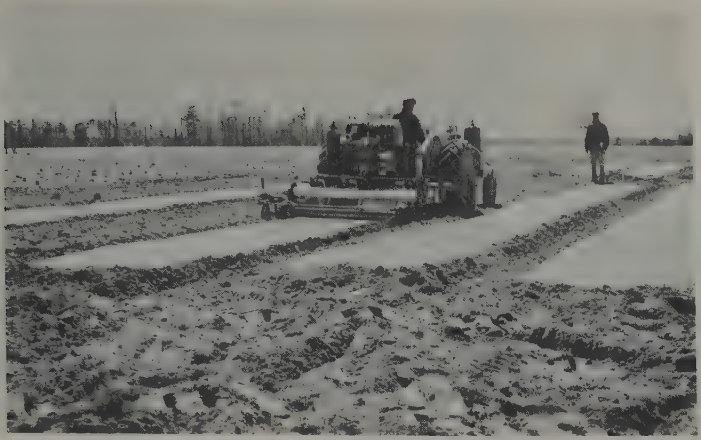

FIGURE 1. Application rig dispensing soil fumigant, by injection or vaporization at soil surface, and laying of polyethylene cover in one continuous operation. Note alleyways which were treated manually with MC-2.

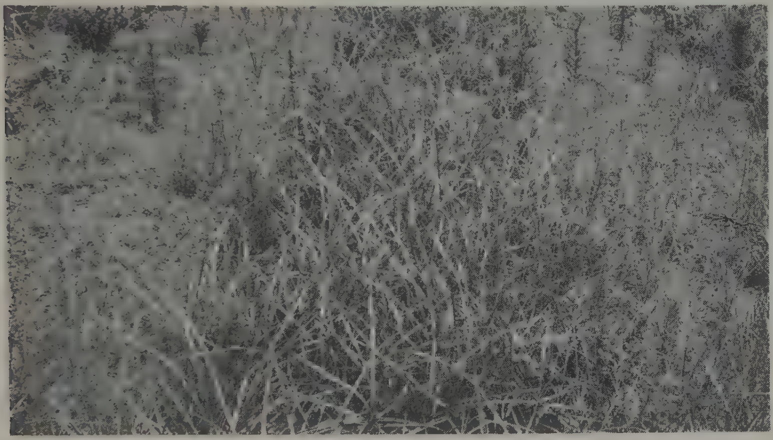

FIGURE 2. Weed development in a nonfumigated plot showing density of yellow nut sedge.

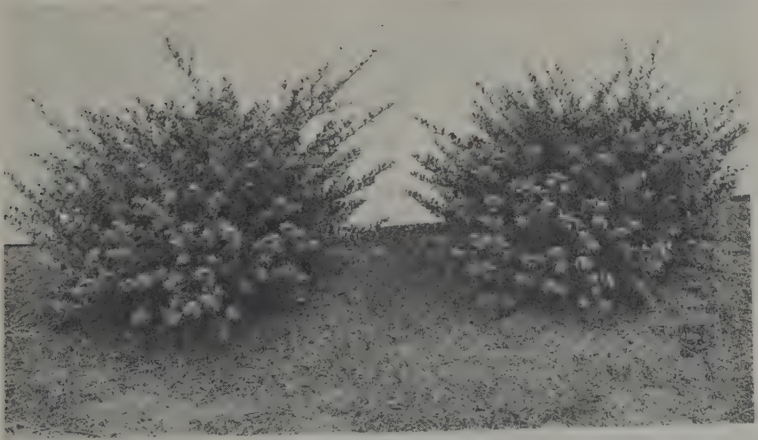

FIGURE 3. Ilex crenata convexa grown for 24 months in a plot treated with Trizone at 200 lb/A. Note uniformity and compactness of the plants.

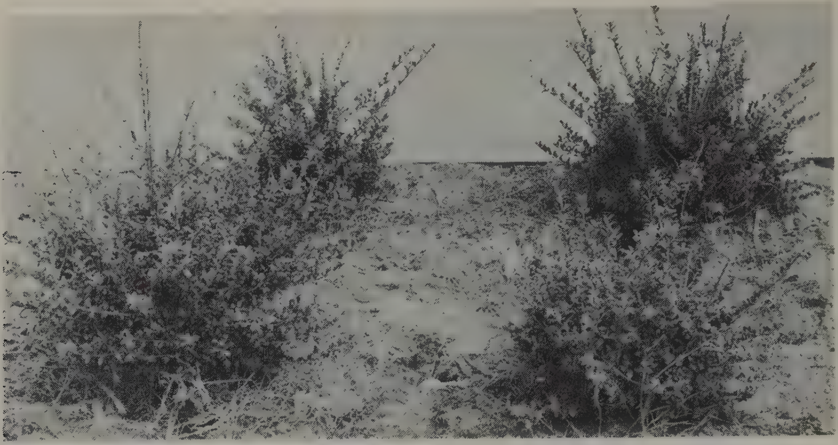

FIGURE 4. Ilex crenata convexa grown for 24 months in nonfumigated plot.

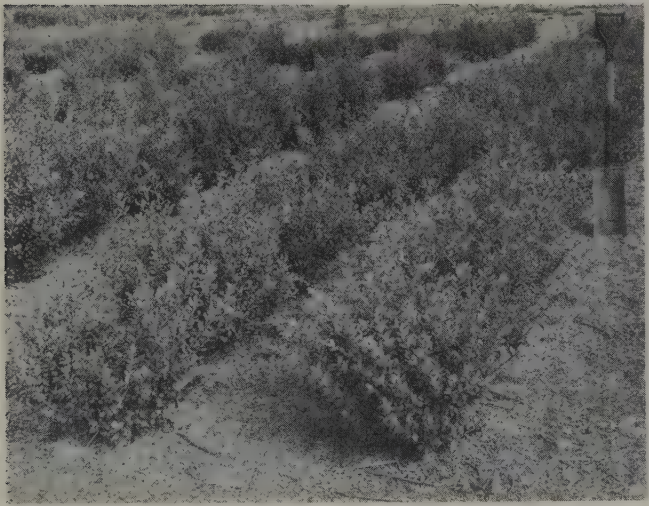

FIGURE 5. Ilex crenata rotundifolia grown for 24 months in a plot treated with Brozone at 434 lb/A. Note the uniformity in shape and size of plants.

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PATHOGENICITY OF STEMPHYLIUM LOTI ON SEVERAL BLUE LUPINE BREEDING LINES RESISTANT TO S. SOLANI AND S. BOTRYOSUM[1]

Homer D. Wells, Ian Forbes, Jr., and John R. Edwardson[2]

Abstract

Stemphylium loti is highly pathogenic on breeding lines of blue lupine (*Lupinus angustifolius*) resistant to two other *Stemphylium* species (*S. solani* and *S. botryosum*) which cause extensive damage to currently grown commercial varieties of blue lupines.

INTRODUCTION

Previous investigations (1, 5, 6) have shown that both *Stemphylium solani* Weber and *S. botryosum* Wallr. frequently have caused severe damage to commercial varieties of blue lupine (*Lupinus angustifolius*). The discovery of 13 strains of blue lupine resistant to gray leaf spot caused by *S. solani* was reported by Forbes, et al. in 1957 (2), and breeding lines previously found resistant to *S. solani* were also shown to be resistant to virulent strains of *S. botryosum* in 1961 (Wells, et al. (5) and Edwardson, et al. (1)). Graham and Zeiders (4) reported that *S. loti* Graham from birdsfoot trefoil (*Lotus corniculatus*) was pathogenic on blue lupine in their inoculation trial.

The present study was conducted in the spring of 1961 to determine whether resistance to *S. loti* exists in unrelated blue lupine breeding lines carrying resistance to *S. solani* and *S. botryosum.*

MATERIALS AND METHODS

Two isolates of *S. loti* (60-16 and 60-17) that Graham and Zeiders (4) had determined to be pathogenic on Borre sweet blue lupine were used as the source of *S. loti* inoculum. One isolate of *S. solani* (56-36) and one of *S. botryosum* (60-23-A) were also used as sources of inoculum. All blue lupine and fungus entries reported in Table 1 were replicated twice in a completely randomized experimental design. The experimental techniques described by Forbes, et al. (3) were used in the pathogenicity test. Since *S. loti* has not been reported from Georgia, the inoculated plants were maintained under quarantine in an east window in the laboratory and all contaminated materials and *S. loti* cultures were autoclaved after completion of the experiment.

RESULTS

The data reported in Table 1 show that all blue lupine entries having resistance to *S. solani* and *S. botryosum* were highly susceptible to *S. loti*. The Blanco sweet blue lupine control was susceptible to all three species of *Stemphylium*.

DISCUSSION

The fact that numerous unrelated blue lupine breeding lines resistant to *S. solani* are also resistant to virulent strains of *S. botryosum* suggests that the gene or genes which confer resistance to *S. solani* in these breeding lines also confer resistance to virulent strains of *S. botryosum*. It is unfortunate from the breeder's standpoint that the disease protection afforded by this gene or genes does not also include protection against damage by *S. loti* However, the absence of any reports of *S. loti* on blue lupine in southeastern United States indicates

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[1] Cooperative investigations at Tifton, Georgia and Gainesville, Florida of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, the University of Georgia College of Agriculture Experiment Stations, Coastal Plain Experiment Station, and the Florida Agricultural Experiment Station, Gainesville. Published with the approval of the Director of the Coastal Plain Experiment Station as Journal Series Paper No. 92.

[2] Pathologist and Research Agronomist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Tifton, Georgia; Associate Agronomist, Florida Agricultural Experiment Station, Gainesville, Florida, respectively.

Table 1. Reactions of gray leaf spot resistant blue lupine lines and Blanco blue lupine to two isolates of S. loti, one isolate of S. solani, and one isolate of S. botryosum 7 days after inoculation.

Blue lupine line	Reactions[a] to S. loti 60-16	Reactions[a] to S. loti 60-17	Reactions to S. solani (56-36)	Reactions to S. botryosum (60-23-A)
S-1	+	+	-	-
S-4	+	+	-	-
S-5	+	+	-	-
S-6	+	+	-	-
S-7	+	+	-	-
S-8	+	+	-	-
S-9	+	+	-	-
S-10	+	+	-	-
WH-1	+	+	-	-
S-13	+	+	-	-
Blanco	+	+	+	+

a - = no infection.
+ = severe damage resulting in defoliation and death of seedlings.

that this fungus may not be adapted to, or at least is not present in, this region. Possibly the fungus may never threaten blue lupine culture in the South. Nevertheless, should the fungus become a problem in the South, screening techniques are available for isolating resistance to S. loti in blue lupine populations. Genetic studies are underway to determine the number of genes controlling resistance to the gray leaf spot complex in the ten unrelated breeding lines listed in Table 1. If several different genes are identified, the combination of two or more of them in a single line might result in resistance to S. loti.

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THREE COWPEA MOSAIC VIRUSES FROM GLADIOLUS

Philip Brierley and Floyd F. Smith[1]

Summary

A cowpea strain of bean yellow mosaic virus was isolated from three gladiolus cultivars. Chenopodium amaranticolor distinguished this cowpea strain from a common strain. Physical properties confirm those recorded by Anderson, but aphid transmission failed. Two undetermined viruses from two gladiolus cvs. distorted cowpea leaves but were difficult to transfer from cowpea. These two were not seed-borne.

Using Blackeye cowpea (Vigna sinensis) as a test plant for ringspot or cucumber mosaic viruses, we detected a cowpea strain of bean yellow mosaic virus (BYMV) in the gladiolus cvs. Elizabeth the Queen from Florida and in Valeria and Friendship gladioli from New York. The Friendship cv. supplied from the Ornamentals Laboratory, Farmingdale, New York, by Arthur Bing proved free from ringspot and cucumber mosaic viruses, but 53% of 131 plants indexed yielded a cowpea strain of BYMV and the remaining 47% yielded common strains of this virus. Two other cowpea viruses were isolated from Spic and Span from New Jersey and from Friendship from New York.

A COWPEA STRAIN OF BEAN YELLOW MOSAIC VIRUS

In gladiolus the cowpea strain of BYMV caused mild mottling in young leaves and mild flower breaks similar to those produced by common strains. The cowpea strain seemed no more damaging to gladioli than common strains, but it proved confusing in indexing gladioli for ringspot viruses. The titer of this cowpea strain was lower in gladiolus than in cowpea or in Pinto bean (Phaseolus vulgaris) and lower in post-flowering leaves of gladiolus than in pre-flowering.

Blackeye cowpea seedlings inoculated from greenhouse-grown gladioli in August developed partial stem necrosis and then produced mottled compound leaves (Fig. 1, C). Inoculated primary leaves occasionally developed light-green local lesions (Fig. 1, A). Inoculations from gladiolus of similar age in October and November nearly all produced local lesions and with few exceptions killed Blackeye cowpea before compound leaves were formed. Transfers of the virus from cowpea or from Pinto bean to cowpea regularly produced green-spot local lesions (Fig. 1, A) and killed the plants. Such local lesions sometimes became purple-ringed, simulating the response to ringspot viruses. Older cowpeas inoculated on the compound leaves developed similar local lesions on these, as well as necrotic stems.

Pinto bean plants inoculated from cowpea in August developed vein necrosis, epinasty, and mild yellow mottling. Bountiful beans responded with epinasty and yellow mottling. In October symptoms in Pinto were more pronounced and stem necrosis sometimes appeared.

Chenopodium amaranticolor responded to common strains of BYMV with yellow-spot local lesions after 6 to 10 days. Infection spread later along branch veins (Fig. 1, E) and some spots and streaks turned red. When the cowpea strain was in low titer the Chenopodium reaction was like that of common strains. From high-titer host plants such as cowpea or bean, and sometimes from gladiolus, this indicator developed a spotty yellow mottling (Fig. 1, D) specific for the cowpea strain. Two common strains failed to duplicate this mottling even when transferred in high titer from Pinto bean.

Physical properties of the cowpea strain, determined in November from Pinto bean to Chenopodium, duplicated Anderson's (1) findings: Heating 60° to 65° C, dilution between 1:10,000 and 1:50,000, and aging 1 to 2 days at about 25°. Tests from necrotic Blackeye cowpea to cowpea and Chenopodium gave the same end points in Chenopodium, but the dilution end point in cowpea fell to 10^{-3}.

Attempts to transmit this cowpea strain from Blackeye cowpea, from Pinto bean, or from Friendship gladiolus to Blackeye cowpea in three tests with Myzus persicae Sulz. or in one test with Macrosiphum pisi Harris all failed. The peach aphid readily transmitted a common

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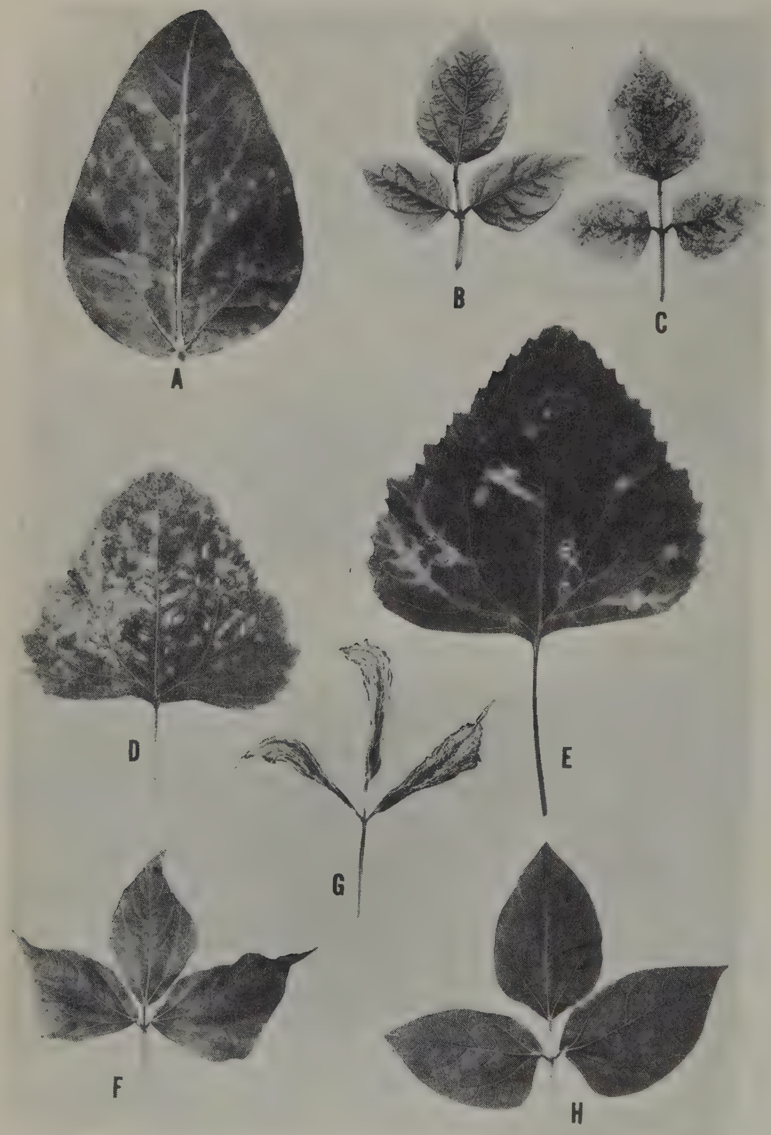

FIGURE 1. Symptoms of cowpea viruses. A-E. Bean yellow mosaic strains. A -- Local lesions of a cowpea strain in Blackeye cowpea leaf. B -- Normal, and C -- infected compound leaves of Blackeye cowpea. D and E. Chenopodium reactions, D -- to a cowpea strain, E -- to a common strain of BYMV. F-- Mild mosaic. G -- Severe mosaic. H -- Normal Blackeye cowpea.

strain from gladiolus to gladiolus and it transmitted cowpea strains in Anderson's tests (4). The prevalence of the cowpea strain in the Friendship cv. suggests that some effective vector must be active.

SEVERE AND MILD COWPEA MOSAIC VIRUSES FROM GLADIOLUS

In June 1961, Spic and Span gladiolus plants from New Jersey were indexed on Blackeye cowpea, Chenopodium, and Kentucky 35 tobacco to determine whether stubby spikes were correlated with tomato ringspot virus infections. No ringspot viruses were detected, but inocula from 2 of 19 plants produced mottling and distortion of compound leaves of cowpea (Fig. 1, F, G). No symptoms appeared in Chenopodium or tobacco. In July 1961, the same symptoms developed in cowpea 6 days after testing leaves from a stubby spike of Friendship from New York. Three Blackeye cowpeas developed symptoms of three degrees of severity, including the severe (Fig. 1, G) and mild (Fig. 1, F) types illustrated. These cowpea viruses failed to infect Pinto bean.

Because some colleagues attributed these leaf-distorting symptoms to 2,4-D injury, we repeated the transfer from Spic and Span to Blackeye cowpea. Nine of nine inoculated cowpeas developed severe distorting mosaic (Fig. 1, G) after 6 days, as soon as compound leaves developed. Symptoms on the second pair of compound leaves were milder and similar to Fig. 1, F. This test and others indicate that the milder mosaics here accompany the severe. On transfer from cowpea to cowpea symptoms were slow to appear. Mild mosaic symptoms appeared 1 month after inoculation from cowpea with severe mosaic symptoms. Later some plants developed narrow and distorted leaves approaching the severe symptom type. The mild cowpea mosaic virus was isolated from the original Spic and Span source plant in a second trial, but transfer from mild cowpea mosaic to cowpea failed. The ease of transfer of these viruses from gladiolus to cowpea and the difficulty of transfer from cowpea to cowpea imply that these are gladiolus viruses rather than cowpea viruses. The three gladioli that supplied these viruses had stubby spikes. However many other gladioli with stubby spikes yielded only strains of BYMV. Corms infected by Stromatinia gladioli (Drayt.) Whet. and even corms with multiple shoots may produce stubby spikes; hence the association of short spikes with these viruses may be coincidental.

We failed to transmit the severe or the mild mosaic virus from Blackeye cowpea to cowpea with the aphids Myzus persicae, Macrosiphum pisi, or Aphis gossypii Glov. One hundred fifty two seeds of Blackeye cowpea from mild mosaic parents and 315 seeds from severe mosaic parents produced no evidence of seed transmission of either virus.

Strains of BYMV, including one necrotic in Blue Lake bean, were isolated from gladiolus by McWhorter, Boyle, and Dana (6) in Oregon. Anderson (1, 2, 3, 4) studied cowpea viruses in Florida, identifying cucumber mosaic virus and BYMV, and Corbett (5) showed one of these cowpea viruses serologically related to known BYMV. Cowpea strains of BYMV have not previously been reported from gladiolus. Common strains of this virus fail to infect cowpea. The curious leaf-deforming cowpea mosaic viruses isolated from gladioli bear no resemblance to the viruses previously described from cowpea.

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MECHANICAL TRANSMISSION OF VIRUS FROM DISEASED PEAR TREES TO HERBACEOUS HOSTS

Hillegonda J. Pfaeltzer[1]

Summary

A virus was mechanically transmitted from leaves of pear trees with stony pit disease to *Sesbania exaltata* and *Cyamopsis tetragonoloba*. These isolates could not be maintained on herbaceous hosts. From leaves of pear trees with ring mosaic disease a virus was transmitted mechanically to *Chenopodium quinoa* plants. These isolates could be maintained by repeated transmission from *C. quinoa* to *C. quinoa*. A relation between the two virus diseases mentioned and the virus isolates obtained in herbaceous hosts is not yet demonstrated.

Although a pear tree is healthy looking, it may still be virus-infected. A piece of a tree suspected of being infected with a certain virus must be grafted or budded to one or more species of indicator plants; then, if the indicator plants remain free from virus symptoms the pear tree may be presumed free from any of the known virus diseases. At present only woody indicator plants are available for the virus diseases of pears, a method of testing that is both slow and costly. In an attempt to find a quicker method, therefore, we have been searching for herbaceous indicator plants. This paper reports the successful transmission of virus from pear trees to herbaceous hosts.

In 1961 we received leaf samples taken from pear trees with ring mosaic (Fig. 1) and stony pit virus diseases. The samples were kept in plastic bags at ± 3° C.

Stony Pit Disease: Leaf samples taken in May from four trees were separately ground and inoculated on a number of test plants (cucumber, White Burley tobacco, *Chenopodium amaranticolor*, *C. foetidum*, *C. quinoa*, *Phlox drummondii*, and *Sesbania exaltata*). In two cases a few local lesions were observed on the inoculated cotyledons of the *Sesbania* plants (Fig. 2), and in a third case a faint spot was found on the leaf of a *C. quinoa* plant. Transmission from these lesions to White Burley tobacco, *C. quinoa* and cucumber was unsuccessful. Some weeks later the same sources were used for inoculations to *Sesbania exaltata* and *Cyamopsis tetragonoloba*. Two of the samples produced a few local lesions on *Sesbania* and one produced also two vague local lesions on the cotyledons of a *Cyamopsis* plant. Inoculation on *C. quinoa* from the *Cyamopsis* plant resulted in one local lesion, but we failed to get any further virus transmissions with this material.

Early in July we received additional samples taken from other pear trees infected with stony pit disease. These samples were tested on *Cyamopsis tetragonoloba*, *Chenopodium quinoa*, *C. amaranticolor*, *C. foetidum* and White Burley tobacco. Four samples produced local lesions on the *Cyamopsis* cotyledons and three also produced local lesions on White Burley. We were again unable to maintain these isolates on herbaceous plants.

Ring Mosaic of Pear: In March, buds were taken from two severely diseased pear trees. The buds were cleaned free of scales, ground in a few ml of a 2 1/2% nicotine solution and the sap inoculated on *C. quinoa* leaves and cucumber cotyledons. One of the samples was also inoculated on petunia, *C. foetidum* and *Ammi majus*. A few local lesions were observed on *C. quinoa* inoculated with one sample and on *C. foetidum* inoculated with the other. Both isolates were transmitted further to cucumber, White Burley tobacco, tomato, *C. foetidum* (Fig. 3) and *Nicotiana rustica*. All the hosts reacted with necrotic local lesions but did not become systemically infected. *Phaseolus vulgaris*, *Phlox drummondii*, petunia, *Nicotiana glauca* and *N. glutinosa* also reacted with necrotic local lesions.

In May we received five leaf samples and tested them on a host range similar to that used previously. Local lesions developed on the inoculated leaves of *C. quinoa* (Fig. 4) and on the cotyledons of one *Sesbania* plant. Virus could be transmitted from *Sesbania* to *Sesbania*. Two other isolates were transmitted several times from *C. quinoa* to *C. quinoa*. We did not find other host plants for these isolates.

In October of the same year there were still a few green leaves in the leaf samples of pear which had been kept in a cold room since the previous spring. We used these leaves for testing on *C. quinoa*. All plants inoculated produced local lesions. The virus was transmitted from *C. quinoa* to *C. quinoa* and to *C. amaranticolor* (Fig. 5, A and B). Both species showed

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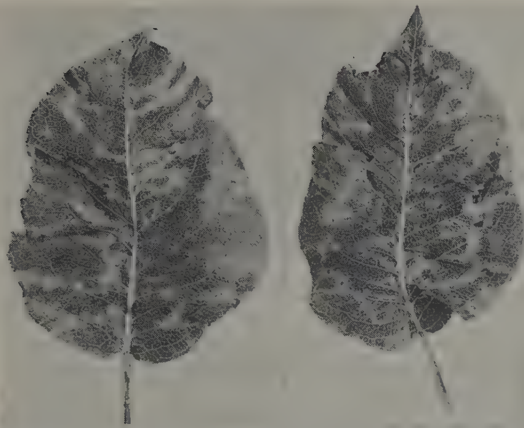
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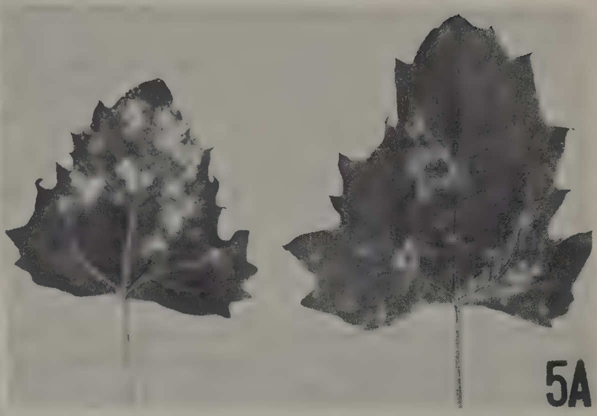
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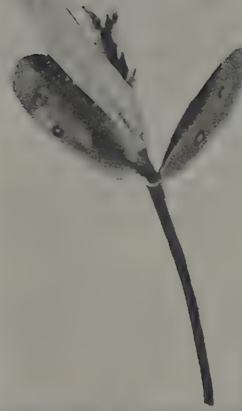
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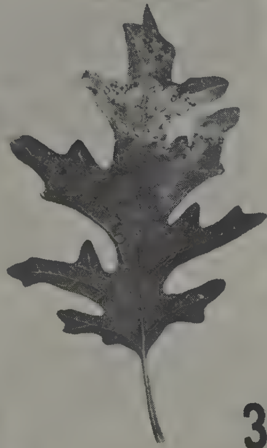
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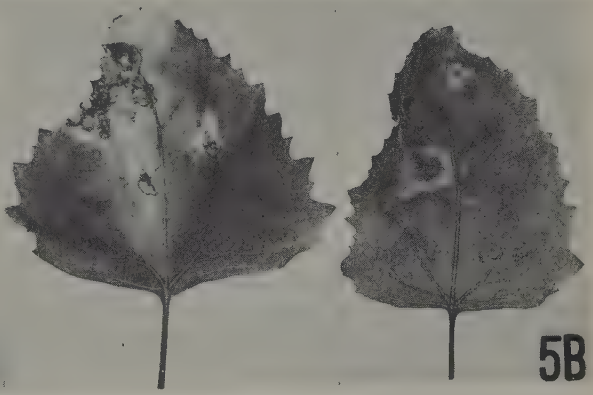
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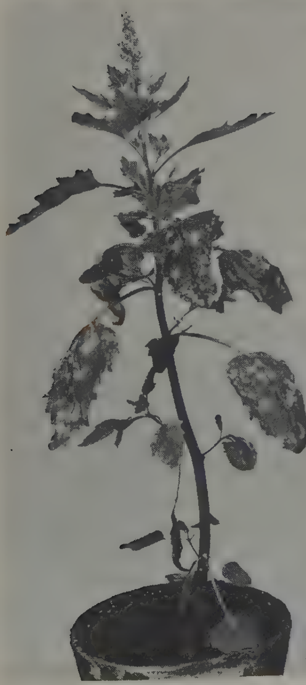
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FIGURE 1. Leaves of Beurré Hardy showing symptoms of ring mosaic.

FIGURE 2. Local lesions on the cotyledons of *Sesbania exaltata* inoculated with sap from leaves of pear trees affected with stony pit.

FIGURE 3. Local lesions on a leaf of *Chenopodium foetidum* inoculated with sap extracted from buds taken from a pear tree affected with ring mosaic.

FIGURE 4. Symptoms on *Chenopodium quinoa* produced by inoculation with sap from leaves of pear showing symptoms of ring mosaic.

FIGURE 5. Symptoms on *C. quinoa* (A) and *C. amaranticolor* (B) inoculated with sap from pear leaves showing ring mosaic symptoms. Local lesions, left and systemic symptoms, right.

local lesions and systemic ring and line patterns. We were not successful in transmitting the virus to cucumber, White Burley, petunia, *Sesbania* or *Nicotiana rustica*.

The results of these preliminary tests show that it is possible to transmit virus from pear trees to herbaceous hosts. We do not yet know if these isolates are the causes of the particular pear diseases concerned. We shall be sure of that only when it is possible to induce symptoms in healthy pear trees by inoculating them with virus from the herbaceous hosts. We also do not yet know if our results are consistently reproducible.

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FOMES ANNOSUS: A REPORT ON THE PRODUCTION OF CONIDIA IN NATURE AND OTHER STUDIES IN VIRGINIA

C. L. Morris and K. A. Knox[1]

Summary

Scattered colonies of Fomes annosus bearing fruiting conidial heads were noted consistently on exposed stump surfaces in late February. Trees were cut in early November of the previous year. Ammonium bifluoride and urea appeared suitable materials for stump protection -- at least during the autumn months.

Windborne spores from the conks of the root-rotting fungus Fomes annosus have generally been considered the main source of inoculum for establishing new infections. The role of conidia in establishing new infections has not been fully evaluated.

Rishbeth (1, 2) felt that it was unlikely that conidia played an important part in the dispersal of the fungus, although he had observed them very occasionally in nature in bark beetle tunnels and under the bark of infected trees at ground level. He later reported noting conidia fruiting on a stump of Douglas fir which had been covered with branches after felling.

The authors have recently been evaluating various materials for stump protection against Fomes annosus. On February 28, 1962, eight discs were cut from the tops of untreated stumps resulting from a second thinning in early November 1961 in a 26-year-old loblolly pine plantation in the Central Piedmont. When these discs were examined in the laboratory several hours later, small colonies bearing conidiophores and conidia were noted on the top surface of all eight. When these sections were incubated in a closed polyethylene bag for 12 hours at 75°F, numerous additional asexual spores were found on the stump surface. Conidia on the stump tops are produced only from areas of exposed wood not covered with resin.

An additional four discs from stumps resulting from a later cut in December-January 1961 in the same plantation were collected on February 28. None of these discs showed any asexual spores of F. annosus at the time of collection, but all showed evidence of infection by producing the asexual spore stage on both top and bottom of the disc when incubated for 3 to 4 days in the laboratory.

Although the conidiospores originally present on the stump tops were not tested for viability, the rapid production of additional asexual spores following only a brief period of higher temperatures indicates that under certain favorable conditions in nature, conidia produced on stump tops and other infected material may serve as an important source of inoculum.

To determine whether F. annosus has been successful in invading freshly cut stumps, suspect stumps can be sampled following exposure by cutting a disc 1 to 2 inches thick and incubating it in a closed polyethylene bag for 6 to 10 days at room temperature. Time elapsed from germination to conidial production on freshly cut pine discs used for spore sampling has consistently taken 6 days under these conditions. Discs used for spore sampling or discs from stumps cut a week or less should be handled under aseptic conditions to prevent chance infection on the underside of discs at time of collection. Atomizing 95% of alcohol on the undersides of discs at time of collection following the exposure period has proved a satisfactory method.

When stumps exposed several months to infection are sampled, the underside of the disc need not be sterilized at time of collection. If infected, conidia will appear within 2 to 4 days following collection when incubated as described previously, whereas conidia produced from chance contamination at time of collection require a minimum of 5 to 6 days to develop. Possible exception to this method: When outside temperatures have been consistently low enough from time of cutting until collection to prevent germination and growth of invading spores.

Small pine wood discs 1/2 to 1 inch thick have been used extensively by a number of investigators in Europe and the United States for spore trapping to determine presence and comparative intensity. In Virginia studies, freshly cut discs of loblolly pine 3 to 4 inches in diameter are utilized for such studies and also for preliminary screening of various stump protectants. For spore trapping, discs are prepared as recommended by Rishbeth (3) and exposed from 3 to 7 hours, resting on heavy wrapping paper. On collection, the bottom of the disc is sterilized by liberally atomizing 95% ethyl alcohol on that face. After the alcohol is allowed to evaporate for several seconds, the disc is re-inserted into the plastic bag in which it was transported to the exposure area and returned to the laboratory for incubation.

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[1] Virginia Division of Forestry, Charlottesville, Virginia.

Conidiophores are produced first on the exposed surface of the disc within 6 days if infection has occurred. For a 1-inch disc, an additional 5 days are required for the fungus to grow through the disc and produce conidia on the opposite side.

When discs for spore trapping or treatment studies are being selected, sections from wood that might have a high percentage of heartwood should be avoided. Spores of F. annosus apparently failed to germinate and grow on heartwood of Virginia pine used in some early studies, but grew well on the thin outer layer of sapwood.

Ideally, discs should be cut from new bolts and used within a week. Bolts 2 1/2 to 3 feet long with the cut ends covered with polyethylene bags, which were stored as long as 6 weeks in the refrigerator at 44°F and then sectioned into discs for immediate exposure, still supported satisfactory growth of F. annosus following exposure. Discs cut and stored individually in the refrigerator for 3 weeks were less satisfactory, since successful colonization by annosus was considerably slower, possibly reduced.

PRELIMINARY RESULTS OF STUMP TREATMENT

On November 1, 1961 a series of six treatments were established on two adjacent 1/5-acre plots on the Buckingham-Appomattox State Forest in Piedmont, Virginia. All treatments were applied within 1 minute after cutting. Infection hazard was extremely high at time of treatment. Stumps were sampled February 28 and March 16, 1962 and incubated in the laboratory for 4 days to determine effectiveness of the various protectants (Table 1).

Table 1. Effect of treatment of loblolly pine stumps on colonization by Fomes annosus.

Material	Concentration	No stumps sampled	No. infected	% protection
Unico Brand Creosote	-	5	3	40
Alcatraz Brand Creosote	-	6	4	33
Urea	2 lb 45% nitrogen/gal. H_2O	6	1	83
Ammonium bifluoride	5% in H_2O	6	1	83
Soil cover	-	6	6	0
Check	-	5	5	0

All treatments except the soil covering considerably reduced the number of successful colonies of F. annosus when compared with the checks. Discs from both check stumps and stumps covered with soil were, in all cases, 50 to 70% covered with annosus colonies following the 4-day incubation period. In contrast, discs from stumps treated with the other materials which showed infection averaged only one colony per disc following incubation. Both urea and ammonium bifluoride encouraged rapid invasion of the stump top by such organisms as Penicillium spp., Gliocladium sp., Trichoderma spp., Botrytis spp., and occasionally Cunninghamella sp. and others, whereas creosote delayed invasion by other fungi. The preceding field test results confirmed preliminary studies utilizing test discs and the same materials.

Because tests in mid-winter with the above materials and other creosotes have not yet been evaluated, the Virginia Division of Forestry is recommending stump treatment with urea or ammonium bifluoride during the warmer months, and a suitable creosote conforming to Federal Specification TT-C-655 during the winter months. Ammonium bifluoride is more difficult to obtain and extremely corrosive to metal. Urea, which is readily available as a fertilizer, appears the best choice at this time for use during warmer weather (April through early November). Stump treatment should be made immediately following cutting to allow maximum penetration of the oil or water-borne protectant.

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PRELIMINARY INVESTIGATIONS ON BIOLOGICAL CONTROL OF GRAY MOLD (BOTRYTIS CINEREA) OF STRAWBERRIES

Dibya Deo Bhatt[1] and E. K. Vaughan[2]

Abstract

Gray mold rot (Botrytis cinerea Pers.) of strawberry usually is controlled by preharvest application of fungicides. When greenhouse strawberries were inoculated at late bloom stage with spore suspensions of Cladosporium herbarum, Pullularia pullulans or Penicillium sp. inhibition of Botrytis rot was 42, 31 and 4%, respectively. When spore suspensions of Cladosporium were sprayed on strawberries in the field, using the same equipment used for spraying fungicides, the percentage of rotted ripe fruits was not reduced, but the yield of marketable berries was significantly increased. The increase is attributed to prevention of blossom blight and green fruit rot by Cladosporium.

INTRODUCTION

Botrytis cinerea Pers., a weakly parasitic organism, usually invades healthy strawberry fruits only after an initial period of growth as a saprophyte on dead or senile floral organs (5). Brown and Montgomery (2) first suggested that in winter lettuce older leaves provide a favorable habitat for the establishment of saprophytic antagonists that prevent infection of young leaves by Botrytis cinerea. Newhook (3) and Wood (6) isolated from dead lettuce leaves a number of bacteria, actinomycetes and fungi which in dual cultures or on detached leaves inhibited growth of Botrytis. However, on prior or simultaneous inoculation of the saprophytic antagonists and Botrytis in the field, no reduction in disease was obtained.

Newhook (4) controlled gray mold (B. cinerea) of greenhouse-grown tomatoes by inoculating fruits at late petal fall stage with suspensions of spores of Cladosporium herbarum or Penicillium sp. He also showed that control of gray mold with fungicidal sprays is obtained at the expense of saprophytic protection, but in the absence of competing organisms saprophytic antagonists such as Cladosporium and Penicillium recolonize, rapidly increase in population and prevent or reduce Botrytis infection.

Bhatt and Vaughan (1) found that a number of fungi that do not cause fruit rot are associated with Botrytis in strawberry fruits and flowers. Isolated most frequently were, in order of decreasing frequency, Cladosporium, Pullularia, Dendrophoma, Gnomonia, Fusarium, Phoma, Penicillium and Pestalotia. All these genera stopped growth of Botrytis in dual cultures on potato-dextrose, yeast extract and strawberry agars, either at the point of contact or at a distance. Of these, Cladosporium herbarum, Pullularia pullulans and Penicillium sp. were used in greenhouse experiments to control gray mold. These three fungi were selected because they 1) are isolated most frequently, 2) are reported to have very little pectolytic activity and 3) form an abundance of inoculum (spores). In cultures all three species also synthesize materials that are inhibitory to Botrytis.

These investigations were conducted to study the practicability of natural control of gray mold by inoculating strawberry flowers and fruits with suspensions of spores of antagonistic saprophytic fungi.

MATERIALS AND METHODS

Strawberry plants (Marshall, Siletz and Puget Beauty) were planted in December in 6-inch pots or No. 5 cans, and kept under fluorescent lights to provide 4 hours of extra daily illumination. Blossoms started opening in the first week of February. On each pedicel two flower buds were selected and the rest removed. Controls, inoculated with Botrytis only, were paired with treated flowers or fruits on the same pedicel. Abnormal flowers or fruits were discarded.

In greenhouse experiments, strawberries were sprayed with spore suspensions of Cladosporium herbarum, Pullularia pullulans or Penicillium sp. at late petal fall or early green fruit stage, and again after 7 to 10 days. Check flowers and fruits on the same plants were sprayed with a 1% glucose solution or with a spore suspension of Botrytis cinerea. To prevent other organisms from becoming established on senile floral parts, treated flowers or fruits were caged or plants were placed inside a small humidity chamber, at the time of the second spraying. At this stage humidity was kept low to discourage infection of fruits by Botrytis. When fruits had reached whitening stage, a heavy suspension of Botrytis spores was sprayed on treated and check fruits. The plants were removed to a mist chamber where high humidity was maintained to provide optimum conditions for infection by B. cinerea. Plants were kept in the humidity chamber for 10 to 14 days before final observations on fruit rot development were made.

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RESULTS

The inhibition of rot was only 2% when Cladosporium was sprayed at early green fruit stage, compared with 42% when this saprophytic antagonist was sprayed at late petal fall stage (Tables 1 and 2). This indicates that 1) saprophytes readily become established when the floral organs are in a senescent stage, 2) Botrytis infection takes place at the late bloom stage, 3) if the antagonistic saprophyte is not already established, fruit rot cannot be prevented, and 4) green fruits are very susceptible to Botrytis infection if proper conditions for growth of the pathogen are provided.

Table 1. Control of Botrytis cinerea by prior inoculation of Cladosporium herbarum on strawberry flowers at late bloom stage.

Treatment	No. of fruits treated	No. of fruits infected by Botrytis	% inhibition of rot
Check (Botrytis only)	105	100	-
Cladosporium (later with Botrytis)	175	86	42

Table 2. Control of Botrytis cinerea by prior inoculation of Cladosporium herbarum on green strawberries.

Treatment	No. of fruits treated	No. of fruits infected by Botrytis	% inhibition of rot
Check (Botrytis only)	58	43	-
Cladosporium (later with Botrytis)	58	42	2

Two other fungi, Pullularia pullulans and Penicillium sp., were also used to inoculate strawberries in the greenhouse. Penicillium was ineffective in preventing Botrytis rot, but a significant amount of control was obtained with Pullularia (Table 3).

Table 3. Control of Botrytis cinerea by prior inoculation of Penicillium and Pullularia pullulans on strawberry flowers at late petal fall stage.

Treatment	No. of fruits treated	No. infected by Botrytis	% inhibition of rot
Penicillium	81	57	4
Check	81	60	-
Pullularia	127	56	36
Check	127	80	-

In these experiments, no attempt was made to sterilize flowers or green fruits before inoculating them with antagonists, thus the possibility of infection by Botrytis at the time of application of antagonists is not precluded. Moreover, after inoculation with Botrytis the plants were kept in a very humid atmosphere. Germination and subsequent infection of fruits by airborne spores may have nullified the natural control provided by the saprophytic antagonists.

Prevention of Gray Mold by Cladosporium in the Field: In 1961, a field experiment was carried out to determine the effectiveness of Cladosporium herbarum, originally isolated from strawberry for preventing gray mold of strawberries. Starting at 50% bloom stage and continuing until 2 weeks before harvest, a heavy suspension of Cladosporium spores in 1% glucose solution with a few drops of spreader sticker was sprayed at the same time and using the same equipment used to apply test fungicide on strawberries. Fungicides were sprayed five times, Cladosporium only three. At regular intervals, flowers and fruits were picked from each of the plots, homogenized in a Waring Blendor and plated in strep PDA (Table 4). Dyrene sharply reduced the number of fungi in berries from treated plots and this difference was maintained until the end of the harvest. Cladosporium was the dominant species in isolations from all three treatments. The total mold count inside ripe surface sterilized strawberries from Cladosporium treated plots was lower than from Check or Dyrene treated plots.

Table 4. Frequency of isolation of fungi from Northwest strawberries at various stages of growth following application of Dyrene and Cladosporium as preharvest sprays[a].

Fungi	TREATMENTS											
	Dyrene[b]				Cladosporium				Check			
	5/16	5/30	6/7		5/16	5/30	6/7		5/16	5/30	6/7	
			A	B			A	B			A	B
Cladosporium	7	102	97	5	459	200	290	5	20	182	310	16
Pullularia	-	3	-	21	20	2	22	3	13	-	43	15
Botrytis	2	4	7	1	12	2	23	-	-	14	33	1
Dendrophoma	-	-	-	-	9	-	66	-	3	-	9	-
Penicillium	1	2	-	-	3	-	-	-	-	1	-	-
Fusarium	-	-	-	-	2	-	-	-	2	-	5	-
Rhizopus	-	-	3	-	-	-	-	-	-	5	-	-
Alternaria	-	1	-	-	-	-	-	-	5	2	-	-
Trichoderma	-	-	-	-	-	-	1	-	-	-	-	-
Aspergillus	-	-	-	-	1	-	-	-	-	-	-	-
Stemphylium	-	-	-	-	-	-	-	-	-	1	-	-
Pestalotia	-	1	-	-	-	-	-	-	-	-	-	-
Gnomonia	-	-	-	2	-	-	-	1	-	-	-	-
Melanconium ?	-	-	-	-	-	-	-	1	-	-	-	-
Total (identified)	10	113	107	29	506	204	402	10	43	205	400	32
Total (unidentified)	56	11	165	4	1938	2796	981	8	509	413	1318	29
TOTAL	66	124	272	33	2444	3000	1383	18	552	618	1718	61

[a] Total of 15 plates (three replications) per 50 flowers and green fruits at a dilution of 1:1000.

A Only 25 ripe berries (unsterilized).

B Only 25 ripe berries (surface sterilized).

[b] Dyrene = 2,4-dichloro-6-(o-chloroanilino)-s-triazine.

All ripe berries from 15-foot test rows (replicated five times for each treatment) were picked on seven different dates and weight of rotted and marketable berries determined at each picking (Table 5). The incidence of fruit rot was highest in the middle of the harvest season and low in the beginning and end. There was very little precipitation in June and percentages of rot were low in all plots. Although conditions were very favorable for blossom blight and green fruit rot development, yield of marketable strawberries from Cladosporium treated plots was higher than from Dyrene treated or Check plots (Table 5).

Table 5. Percentage rot and yield of marketable strawberries from Cladosporium, Dyrene and untreated plots in 1961[a].

Treatment	Percentage of rot	Yield in tons per acre
Cladosporium	4.99	8.38
Dyrene	2.84	6.52
Check	4.81	6.81

[a] Average of five replications.

DISCUSSION

Penicillium sp., which forms the widest zone of inhibition against Botrytis on agar plates, is least effective in preventing Botrytis rot of strawberries in the greenhouse. An organism showing strong antagonism against another on agar does not necessarily behave similarly under natural conditions. The effectiveness of Cladosporium and Pullularia in preventing Botrytis rot of greenhouse-grown strawberries may be partly due to the fact that they are able to grow and compete successfully with other organisms under much drier conditions than Botrytis. If infection by Botrytis is prevented at the petal fall stage, fruit rot at harvest is greatly reduced.

Field experiments demonstrated that Cladosporium can be readily established on senile or deal floral organs of strawberries when applied as preharvest sprays (Fig. 1). Higher yields from Cladosporium treated plots suggested that in these plots blossom blight and green fruit rot were reduced and that as a result there were more fruits to be harvested. Low yields from Dyrene treated plots may have been due to foliage injury, caused by excessive concentration of the spray solution.

Artificial biological control has some chance of success when the pathogen is weakly parasitic and must compete with saprophytic microflora on wounded surfaces or moribund tissue before it becomes an active parasite. However, direct use of one organism against the other by spraying spore suspensions on susceptible plant organs has definite limitations in the control of fungus diseases. Success of this method depends upon providing favorable conditions for growth of the antagonist, which in many cases are favorable also for the pathogen. Several days are required for an antagonist to increase to a level that will prevent infection by a pathogen. A fungicide is effective as soon as it is applied. The antagonist becomes progressively more effective in preventing infection; the fungicide gradually loses its effectiveness.

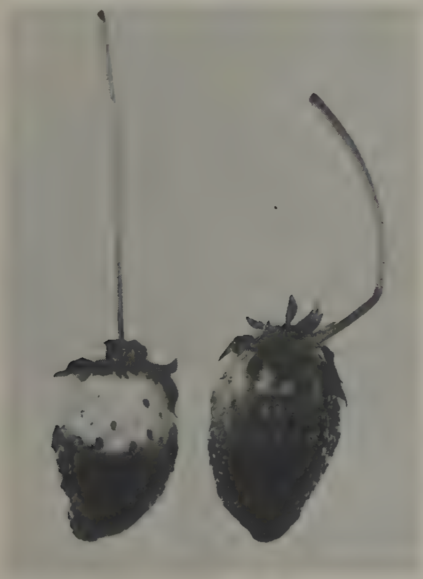

FIGURE 1. Ripe strawberries showing necrotic petals with heavy green mold on berry inoculated with Cladosporium herbarum, left, compared with uninoculated berry at right.

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STYLET-BEARING NEMATODES ASSOCIATED WITH WHITE CLOVERS IN NEW HAMPSHIRE 1960-1961[1]

Tseh-an Chen, R. A. Kilpatrick, and Avery E. Rich[2]

Summary

White clover roots and surrounding soils were sampled for presence of stylet-bearing nematodes from 64 plots at two locations in New Hampshire in 1960-1961. Pratylenchus penetrans was the predominant form. Nematode populations were high in control and dieldrin-treated plots. Methyl bromide provided good control for 3 years after the initial treatment.

Nematode injury to clovers (Trifolium spp.) is common in the southern States. Root injury varies according to the species of nematodes involved. Galls induced by the root-knot nematode (Meloidogyne spp.) are easily recognized. Lesions incited by other species (meadow, sting, and others) are difficult to distinguish from initial fungus lesions. This, plus inadequate study, has probably accounted for the scanty information on nematodes associated with white clover (Trifolium repens) in New England. The objectives of this investigation were 1) to determine the kind of stylet-bearing nematodes associated with roots of white clover, and 2) to compare the population in plots previously treated with a fungicide and an insecticide. Preliminary results have been reported (1).

The plots (10 x 20 feet) selected for sampling had been treated with 1) methyl bromide 1 lb/100 sq. ft.; 2) dieldrin - 50% dust at 8 lb/A; 3) combination of the two chemicals at the above rates; and 4) untreated control. Each treatment was replicated four times. Duplicate plots were treated prior to seeding in 1958 and 1959 at Dover and Northwood, New Hampshire. The original objectives were to study the effects of a fungicide and an insecticide on 1) root rot development; 2) control of clover root curculio (Sitona spp.); and 3) yield. These results were reported elsewhere (2).

Roots and soil were sampled August 8, 1960 and July 17, 1961. Two random samples of roots and soil were taken from each of the 16 plots for the two dates of seeding at both Dover and Northwood. Samples were stored in plastic bags (4° C) until processed; usually within 4 to 7 days.

Roots were examined for discoloration and rotting and then cut into small pieces. Each sample was blotted to remove excess water and weighed. Roots were then distributed on one layer of Kleenex facial tissue supported by a copper wire screen in a Baermann funnel. Enough distilled water was poured into the funnel to cover the root samples. Twenty-four hours later a 50-ml sample was taken. From this sample, at least three 1-ml aliquots were taken and stylet-bearing nematodes were counted. Nematodes were calculated as number per gram of roots.

A 5-g sample of soil surrounding the roots from each of the 64 plots was evenly distributed over the Kleenex facial tissue. Each sample was processed in the manner described above.

Examination of the roots revealed the presence of nematode galls (Meloidogyne sp.) on roots obtained from Northwood. This is the first observation of root knot on clovers from field plots in New Hampshire. No galls were found on roots from Dover. Four explanations may be offered for failing to see galls on roots previously examined: 1) galls were small and easily overlooked; 2) clover roots apparently survive for less than 2 years; 3) short growing seasons; and 4) extreme winter temperatures. Under greenhouse conditions in New Hampshire large galls develop on clover roots.

The results of sampling are illustrated in Table 1. The predominant stylet-form nematode was Pratylenchus penetrans (Cobb) Sher & Allen (identified by A. L. Taylor). In 1960, the average number of P. penetrans was higher in plots treated with dieldrin than in untreated plots.

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and Department of Botany, New Hampshire Agricultural Experiment Station, Durham. Published with the approval of the Director of the New Hampshire Agricultural Experiment Station, as Scientific Contribution No. 287.

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Table 1. Average number of stylet-bearing nematodes found in samples of roots and soil from white clover plots planted in 1958-1959 at two locations in New Hampshire[a].

	1960				1961			
	Roots		Soil		Roots		Soil	
Treatment	1958	1959	1958	1959	1958	1959	1958	1959
Dover								
Methyl bromide (MB)	4[b]	2	1	0	2	12	6	3
MB + dieldrin	7	7	0.2	6	5	24	7	26
Dieldrin	570	57	44	4	161	120	16	61
Control	97	75	7	2	131	171	16	28
Northwood								
Methyl bromide	4	0.7	3	0	4	13	2	2
MB + dieldrin	141	0	8	2	36	13	18	6
Dieldrin	208	693	61	49	547	614	39	61
Control	205	251	16	29	1182	518	44	70

[a] *Pratylenchus penetrans* was the predominant form.

[b] Averages of four replications, expressed as number per gram.

Results were similar from the roots and soil at both locations. More nematodes (*Pratylenchus*) were obtained from Northwood (heavy loam) than were obtained from Dover (fine sandy loam). The results obtained in 1961 were not so consistent as those obtained in 1960. However, higher populations of *P. penetrans* were obtained from dieldrin-treated plots. The data suggest that dieldrin controlled predators of the nematodes, and thereby enabled populations of *Pratylenchus* to increase. Good control was obtained in methyl bromide-treated plots for 3 years following initial treatment.

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POWDERY MILDEW ON STRAWBERRY IN FLORIDA

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In May 1961, the writers were requested to inspect approximately 40,000 climbing strawberry plants, Fragaria chiloensis, variety Red Rocket, then recently imported from Belgium. At least 30% of the plants under inspection showed a peculiar curling of the leaves. Under closer examination, a white powdery growth was observed on the under-surface of affected leaves and stems, but no signs were noted on the berries. Under microscopic examination, the Oidium stage of a powdery mildew was found fruiting profusely on leaves showing this type of leaf curling. The symptoms of the disease correspond to those reported by Anderson (1) for Sphaerotheca macularis (Wallr. ex Fr.) W. B. Cooke. This is believed to be the first report from Florida of this fungus on strawberries.

Fifteen strawberry plants showing infection were planted in clay pots at the Plant Pathology Laboratory, Winter Haven, Florida from May 1961 to February 1962 in order that the course of the disease might be observed and the susceptibility of two commercial varieties commonly grown in Florida (Missionary and Florida 90) tested.

Under greenhouse conditions the Red Rocket variety was quite susceptible to the disease. Signs of the fungus were observed continuously during the course of these studies. There were differences in severity among the Red Rocket plants. Twelve of the 15 plants showed about equally severe susceptibility to the fungus. Two plants showed extremely severe susceptibility, in which some killing was observed. Fungus growth also invaded the top surface of the leaf petioles and fruit. One plant of this variety showed only a few white patches and little leaf curl.

In May 1961, twenty-five strawberry plants each of Missionary and Florida 90 were secured from local growers to test their susceptibility to this fungus. The plants were potted and placed in a shady part of the greenhouse next to and among the infected Red Rocket variety. Darrow, Scott and Goheen (2) reported that strawberry plants growing in greenhouses were more susceptible to infection and that this is probably due in part to the succulence of greenhouse plants as compared with less succulent field-grown plants.

Only a few white patches of the fungus and some slight leaf curling were noted on some plants of each variety from November through February. Although these two varieties are not immune to this disease, apparently they are highly resistant even under greenhouse conditions. This in part agrees with Darrow, et al. (2) who rated Missionary as slightly susceptible. Florida 90 was not included in their studies.

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DIVERSE SYMPTOMS IN PELARGONIUM INFECTED WITH VERTICILLIUM[1]

Frank P. McWhorter[2]

Abstract

A study of Pelargonium cultivars naturally infected and variously inoculated with Verticillium albo-atrum Reinke & Berth. indicates that wilting, hitherto the only symptom reported, is neither the only nor the usual symptom. More usual are bright yellow spotting of leaves, severe necrosis of shoot-terminals or else extreme dwarfing of plants unaccompanied by foliar discolorations or wilting. Data presented show how the yellow spot and necrotic symptoms can be easily confused with symptoms produced by Xanthomonas pelargonii (Brown) Starr & Burk. Verticillium seems to be a far more important factor in the commercial production of geraniums than has been suspected.

OCCURRENCE RECORDS

The importance of Verticillium albo-atrum Reinke & Berth. as a pathogen of Pelargonium has not been generally recognized by producers of geranium cuttings, by florists who market plants grown from cuttings, or by home growers.

Two explanations are probable: 1) There are no popular accounts that present adequate information about the disease. For example, the excellent California Extension Service leaflet (6) devotes nearly half the content to bacterial blight and only seven short lines to Verticillium infection. 2) Verticillium infections may have been noted by professionals and recorded as some other disease. In fact, the hitherto unreported necrotic symptoms so closely simulate those produced by Xanthomonas pelargonii (Brown) Starr & Burk. that a pathologist routinely examining submitted material could easily make an erroneous diagnosis. In 1959, the writer first saw the necrotic form of the Verticillium disease in a commercial planting, judged it to be bacterial blight, and made no cultures. This paper describes local outbreaks of the disease and the diverse symptoms observed in both naturally infected and inoculated species and cultivars of Pelargonium.

During 1959 we grew many seedlings for use as test plants for inoculations with Pelargonium viruses. Also, from Mr. Philip Chandler of the University of California at Los Angeles we obtained several commercial varieties culture-indexed into clones uninfected with X. pelargonii. Following initial growth in new pots, these seedlings and stock-plants were planted during May 1960 into an isolation bed prepared from weedy turf where geraniums had never been grown. The plants grew excellently during the summer of 1960 and no disease of any kind was suspected. During the fall of 1960 the geraniums were transplanted from the isolation bed into pots and placed in a greenhouse. The potting soil, a half-peat, half-soil mixture, from which lime had been inadvertently omitted, had a pH of 5.4. This circumstance is mentioned because malnutrition was suspected when foliar discolorations developed in most of the seedlings and cultured stock during February 1961. Initial pale chlorosis, suggestive of malnutrition, was followed by the specific yellow spotting and extreme die-back symptoms described in this paper. The general appearance of the very sick plants suggested bacterial blight, but that conclusion was negated by their blight-free history and by tests for X. pelargonii. The time between planting in the bed where infection apparently occurred and development of symptoms was 8 months.

During October 1960, cuttings taken from the bed-grown seedlings and stock plants were either rooted in Perlite or grafted on virus-study plants. The rooted cuttings grew well until late April or May, when they developed symptoms like those that had developed 2 months earlier in the plants from which they had been removed. Likewise, the scions grafted to the virus-infected plants developed the disease and both had to be destroyed. The additional 2 months required by the cuttings from infected plants to develop symptoms can be significant when planning control in a commercial planting of geraniums.

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[1] Technical Paper No. 1518. Oregon Agricultural Experiment Station. Cooperative investigation with Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

[2] Plant Pathologist, Oregon State University and Agent, United States Department of Agriculture.

Thanks are due Dr. C. E. Horner for many helpful suggestions and Mr. Robert Halliwell and Mr. Paul Lamptey for assistance in making cultures and isolations.

Cultures of affected plants, made during the summer of 1961, consistently yielded a microsclerotia-forming fungus that had the morphological characteristics of V. albo-atrum on PDA. To test for bacterial blight, isolations were made on both liquid and solid media. Bacteria were frequently isolated, but tests on geranium cuttings held at about 22° C proved none to be X. pelargonii. However, inoculation with a Verticillium isolate produced yellow leaf spots in the leaves of two plants and terminal necrosis in another of the first ten inoculated; these symptoms appeared within 28 days. The remaining seven have grown very slowly during a 4-month period, which is a typical expression of Verticillium infection in "Hortorum" cultivars of Pelargonium. Inoculations of branches of large geranium plants with Verticillium usually produced wilt symptoms such as have been reported in the literature. All tests indicated that Verticillium alone was responsible for the various symptoms described in this paper.

A survey of home and commercial plantings during the summer of 1961 showed Verticillium infections to be frequent in western Oregon geraniums and yellow spotting and necrosis far more prevalent than the classical wilt symptoms.

LITERATURE REVIEW

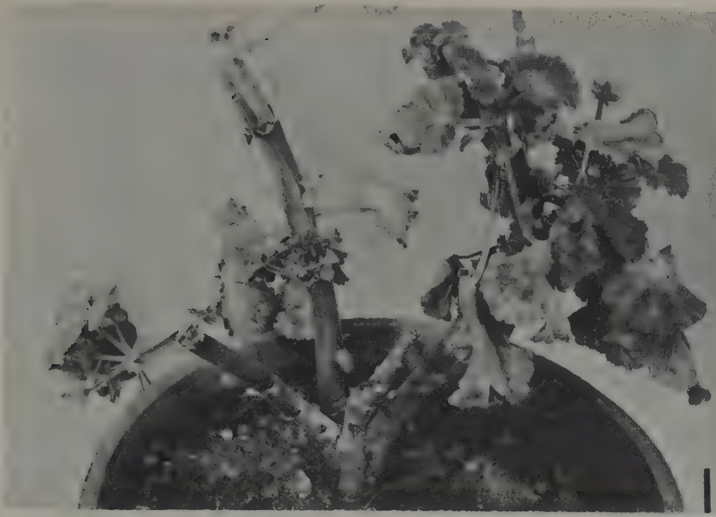
The first records of Verticillium infection of Pelargonium were in 1940. Dimock (3) listed geranium as host but did not describe the symptoms. The first description is by Baker, et al. (1), who in 1940 stated: "Fuchsia hybrida Voss, Pelargonium domesticum Bailey (Lady Washington type), and P. hortorum Bailey from garden plantings in Alameda County have been found repeatedly with typical wilt symptoms and Verticillium albo-atrum has been isolated." In 1952, Torgeson (7) observed the wilt form of the disease in Oregon, isolated Verticillium, and reproduced the wilt form by inoculating large geranium plants. He showed also that Verticillium isolates from raspberry and peppermint readily infected "Hortorum" cultivars and that a peppermint isolate was especially virulent in geranium. In 1958, Nelson and Wilhelm (5) used infected geranium stems in their thermal therapy studies of Verticillium infections and showed that the treatment was inapplicable to geranium because the plant died first. In 1960, Paulus, et al. (6) briefly mentioned Verticillium as the cause of a "typical wilt" occasionally found in California. These few records of Verticillium infections of Pelargonium related only the wilt form of the disease, thereby emphasizing the probability that the other forms here described have appeared elsewhere and were mistaken for X. pelargonii infections.

SYMPTOM COMPLEX

Wilt: Affected plants were noted less frequently from the presence of wilt than from other symptoms. When the lower nodes of large "Hortorum" cultivars are inoculated, the leaves above the inoculation point usually wilt in about 3 weeks. Several leaves along inoculated stems wilt simultaneously and then shrivel, usually without assuming any marked coloration. In one instance, some plants of a seedling clone were planted in a greenhouse and others in a field near the Oregon coast. The coastal plants developed typical wilt; the greenhouse plants developed the yellow spot-dieback symptoms. The circumstances that control symptom expression are unknown.

Yellow Spotting and Dieback: By far the most common sequence of symptoms in our naturally infected experimental planting was: 1) Pale streaks developed along principal veins of fully expanded leaves on one or two principal shoots of large plants, sometimes accompanied by slight wilting (Fig. 3). 2) Portions of the pale streaks changed to bright-yellow spots, which were near the center of the leaf blades or near and on the edge of the leaf (Fig. 4). Also, when vigorously growing plants suddenly developed symptoms, the initial yellow spots often formed at the point where the leaf-blade was attached to the petiole. Spots that formed sectors involving the edge of the leaf simulated spots caused by X. pelargonii. Affected leaves soon dropped and left bare areas of the stems exposed. 3) Shedding of leaves was followed by necrosis of the terminals of affected shoots. Occasionally, however, necrosis began while the leaf spotting was still prominent. Frequently initial necrosis appeared as intensely blackened inflorescences which usually died before they were 3 cm long. 4) Necrosis progressed centripetally from the terminals and often involved as much as 20 cm of a shoot (Figs. 2 and 7). In our plants, leaves never formed in the terminal areas after necrosis had progressed. Thus Verticillium differs from bacterial blight in which partly necrosed stems may recover and produce terminal leaves.

Dwarfing: Development of small, stubby, extremely dwarfed plants was often the only symptom noted in a planting. In a survey of home and commercial plantings, dwarfing was



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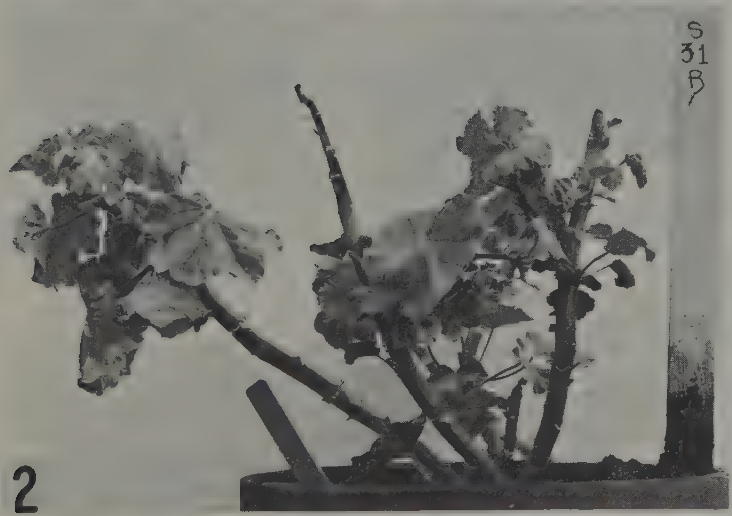

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FIGURE 1. Appearance of geranium plant severely diseased by bacterial blight due to X. pelargonii. (After Munnecke, reproduced with the permission of the California Agricultural Experiment Station)

FIGURE 2. Appearance of geranium plant severely diseased by Verticillium. Note that the general appearance of this plant is similar to the plant diseased with X. pelargonii.

probably the most common expression of the disease. Dwarfed plants have numerous very short nodes, small leaves, no wilt, and usually no yellowing or other discolorations of the foliage. Dwarfed plants were reduced to 1/10th to 1/5th of expected size but usually had comparatively good root systems.

Species Responses: Of our small collection of species, Pelargonium graveolens (fragrant-leaved), P. peltatum (ivy-leaved), P. echinatum (cactus geranium), and the so-called "P. formosanum"[3] were planted in infested soil. The fragrant-leaved plants either became extremely dwarfed or grew fairly well but were marred by clusters of bright-yellow leaves. The ivy-leaved developed die-back symptoms notable for intensely black discoloration of affected parts. All the "formosanum" plants developed bright-yellow coloration of their small, thick, forked leaves and then stopped growing. Only the cactus geranium remained healthy.

DIAGNOSIS

As previously noted by Paulus, et al. (6) vascular discoloration was not observed in plants recognized as Verticillium-infected because of wilt symptoms. No vascular discoloration of the "blue stain" type was observed associated with any of the external symptoms described here, but sometimes there was a slight microscopically notable injury of the vascular cambium accompanied by a faint pink pigmentation. This coloration is present in the lower nodes of stems that have their terminals necrosed but is not sufficiently consistent to be of diagnostic value.

If wilting is conspicuous and continuous when adequate moisture is present, diagnosis of the disease as a Verticillium infection is reasonably certain. Several Fusaria have been reported to cause root rots of Pelargonium but only Verticillium regularly causes wilt while the roots remain comparatively healthy.

Recognition of foliar symptoms is confusing because Pelargonium species and cultivars are often subject to leaf colorations associated with malnutrition or infection by Botrytis and other leaf-spotting fungi. The presence of bright-yellow spots does, however, seem to be specific for Verticillium infection. If the yellow spots involve the edge of the leaf they are readily mistaken for Botrytis infections because Botrytis frequently invades the chlorosed tissue and renders the spots indistinguishable from spots caused by Botrytis alone.

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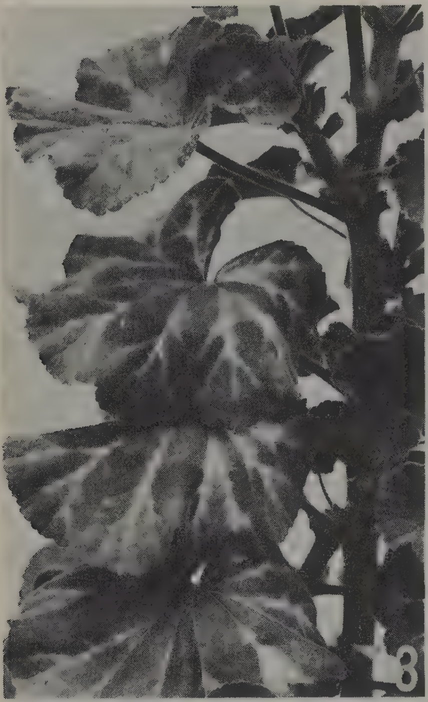
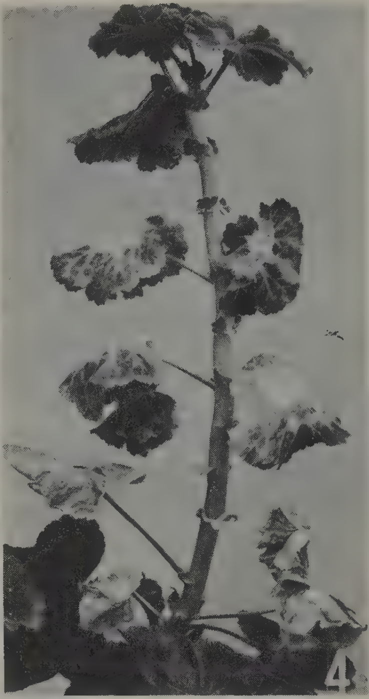
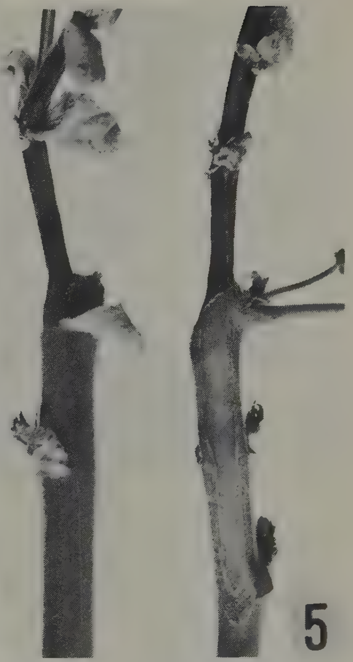
[3] Mr. Holmes C. Miller of Los Altos, California conclusively proved that the novelty "P. formosanum" is not a true species but merely an aberrant seedling among P. hortorum-type parents.







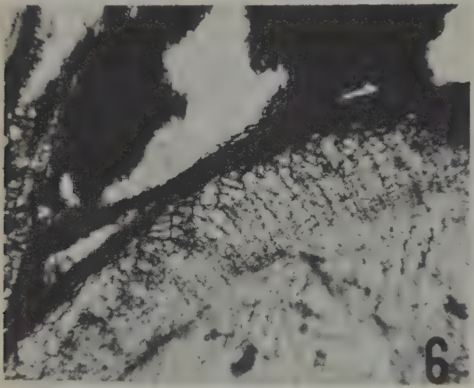


FIGURE 3. Characteristic initial yellowing following natural infection by Verticillium through the roots.

FIGURE 4. Later stage than shown in Figure 3. The white areas are bright-yellow spots that become very pronounced just before the leaves drop.

FIGURE 5. Blackened withered stem terminals characteristic of Verticillium infection. Note the sharply defined wall below necrosed terminal shown in the stem that is cut longitudinally.

FIGURE 6. Longitudinal free-hand section through the base of the necrosed part and the contiguous healthy area to show the cork cambium wall formed against advancing necrosis.

Plants that have groups of yellow leaves on certain branches or scattered in the upper part, whether or not specific yellow spotting has been noticed, are highly suspect and should be tested for Verticillium, especially if the coloration of the leaves is bright yellow.

Isolating Verticillium from infected geraniums is not always easy because so little mycelium is present. Good results were obtained by plating pieces of the yellow spots and segments of the petioles of the leaves in which they occur. Isolations were most consistent from the lower nodes of affected plants, but we have not adequately investigated the relative efficiency of isolating from the stems as compared with isolations from the roots of such plants. Efficacy of isolating from roots should be tested with the specific alcohol-agar medium developed by Nadakavukaren and Horner (4).

When present, die-back symptoms may be considered most specific. Usually the necrosed area of the stem is separated from the healthy by a plate of cork cells formed by a cork cambium that develops across the stem (Fig. 6). Remarkably, the necrosed tissue is almost completely sterile; usually neither living bacteria nor fungi are present and only once have we recovered Verticillium from a necrosed tip. This fact has been confirmed also by microscopical examination. Thus this disease differs from bacterial stem rot where bacteria are notably present. Burkholder (2) recommended making smears of necrotic leaf spots and testing for X. pelargonii by direct microscopical examination. Two other differences between stem rot due to bacteria and Verticillium are: 1) The discoloration of the stem from Verticillium invasion seems darker than from bacteria. 2) Terminals necrosed by Verticillium never seem able to produce leaves again, thus differing from bacterial blight.

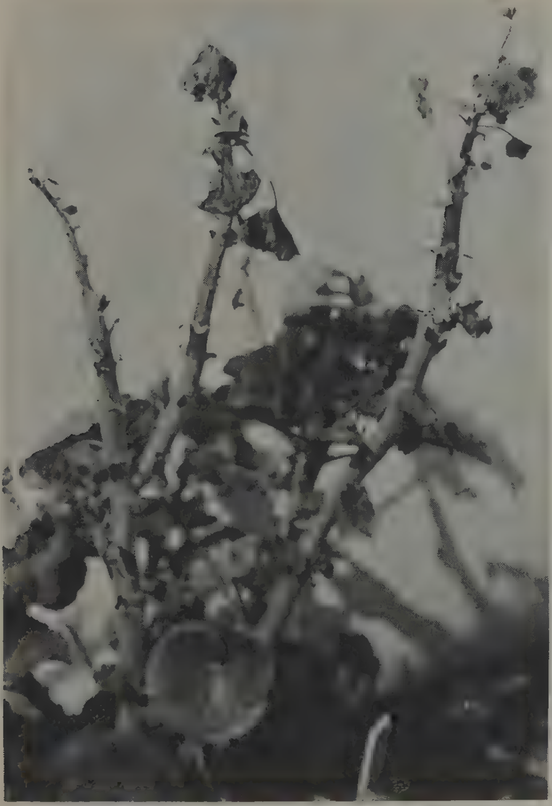


FIGURE 7. Typical appearance of a geranium plant partly recovered from severe infection by Verticillium. This photograph shows the appearance of the plant during August.

NEEDED INVESTIGATIONS

While the usual methods for isolating Verticillium used by pathologists will prove the presence of the fungus in Pelargonium, a dependable procedure is needed for propagators who wish to "culture index" for both X. pelargonii and Verticillium in their greenhouse laboratories. Verticillium can be easily missed.

Since Pelargonium species are readily cloned and easily handled as test plants they should be effective for studies of the basic factors responsible for the large variation in symptoms that characterize Verticillium infections. Among naturally infected geraniums we have noted variations that seemed to be related to nutritional factors. On the contrary, however, when two lots of an infected clone were grown at 50° and 70° F, respectively, slightly more necrosis developed at the low temperature than at the high. Damage to the shoots was always proportionately greater than damage to roots, although the fungus entered through the roots. No data obtained suggest an explanation of the inconsistencies and variations of symptomatology, but Pelargonium does seem a good Verticillium host in which to search for an explanation.

That Verticillium must make infected Pelargonium respond by some profound metabolic change within the plant is indicated by such minute amounts of mycelium producing such severe symptoms. Traces of mycelium in only one or two cells of a thick section of a stem 1 cm in diameter is as much as was found. Usually no mycelium at all could be demonstrated, thus rendering microscopical diagnosis of the disease impractical. Thus easily manipulated Pelargonium plants should be a good host for investigating the elusive "metabolic disturbances" that account for the symptomatology.

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PITTING DISEASE OF BANANA ON THE MARKET

Louis Beraha[1]

Abstract

A pitting disease of banana caused by Piricularia grisea, reported to be of plantation origin in Central and South America, has been found on the Chicago and Milwaukee markets. The disease is characterized by small brown to black, circular, sunken pits which are confined to the skin. Limited studies indicate the fungus may grow equally well over a range of temperatures of from 75° to 89° F.

INTRODUCTION

At various times since 1957, an unusual sunken fruit spot of banana has been observed in Chicago on ripe fruit on the market and in local storage houses. In July 1961, and again in December 1961, this spotting was noted as especially severe by an inspector in Milwaukee, Wisconsin on Cavendish bananas from Honduras. The symptoms agreed with those given by Wardlaw (4) for a pitting disease caused by Piricularia grisea in Central and South America. This is the first report of its presence on bananas shipped from Central America to the United States.

SYMPTOMATOLOGY

The disease is characterized by dry, dark brown to black circular, to nearly oval, depressed spots or pits with a definite outline or border. Non-coalesced pits may measure 1 to 7 mm in size and occasionally have light tan centers. Pitting is confined to the skin only and may extend deeply into this tissue. As observed on the market, pitting appears anywhere on the fruit as it ripens (Fig. 1), but is generally localized on exposed finger stalks and the adjacent fruit surface. The disease is apparently of plantation origin and no spread in storage or on the market has been observed. An estimate of its quantitative occurrence on the different varieties of bananas in marketing channels has not been made.

RESULTS

Isolation of the Causal Agent: Plantings were made on potato-dextrose agar with small pieces of necrotic tissue surface disinfested with $HgCl_2$ (1-1000) and washed in tap water. Among the fungi isolated from these spots, Piricularia grisea was the most prevalent in 1957, 1960 and 1961. The following fungi were found in association with the pitting symptoms at various times: Glomerella cingulata, Fusarium spp., Nigrospora oryzae, Stachylidium theobromae, Pestalotia leprogena, Diplodia theobromae, Phoma sp., and Cladosporium sp. The importance of these secondary organisms to the development of the disease was not determined. Deightoniella torulosa was also isolated from typical raised fruit spots ("speckle") as recently described (1) and is considered distinct from the disease as due to P. grisea.

Description: Conidiophores septate, occasionally branched, sometimes thicker and darker than hyphae; conidia single, attached at the broader end, terminal in scorpioid cymes, ovate to pyriform, hyaline, 2-septate, 23-29 x 9-12μ (Fig. 2). Neither a perfect stage nor a fruiting structure was observed.

Pathogenicity Tests: First attempts to obtain infection using whole green bananas from a local storage house were unsuccessful, owing to the rapidity with which these bananas ripened and the ensuing development of secondary parasites at room temperatures. In other trials, small, immature bananas that had been removed from a tree in the University of Chicago botany tropical greenhouse were used. Inoculations with both spores and mycelium made to both wounded and unwounded disinfested surface areas of the skin were successful. In no case, however, did the pits that developed enlarge beyond 1 to 3 mm in size. Numerous surface pits developed on bananas as the fruits ripened during 8 to 12 days' incubation in moisture-saturated humidity chambers at room temperature (75° to 77° F). Reisolations made from sub-surface

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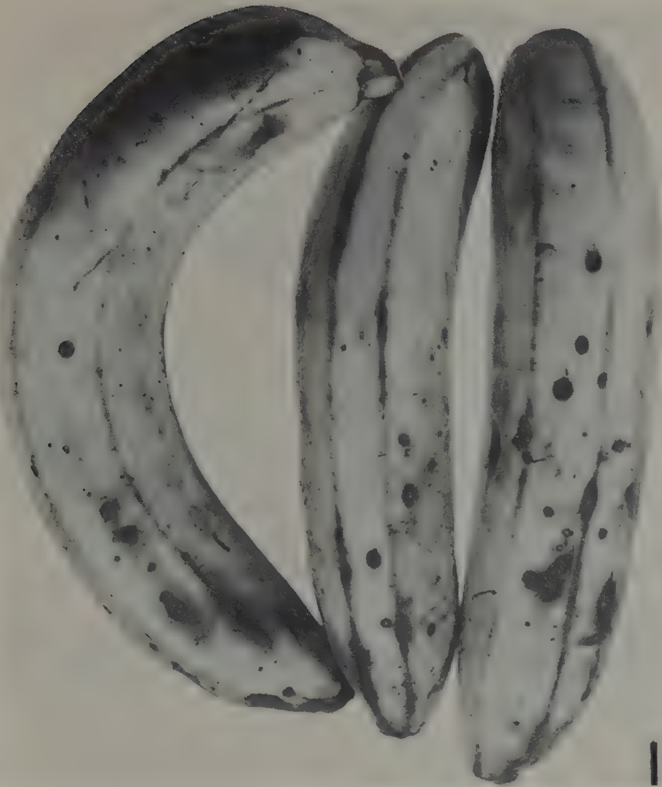

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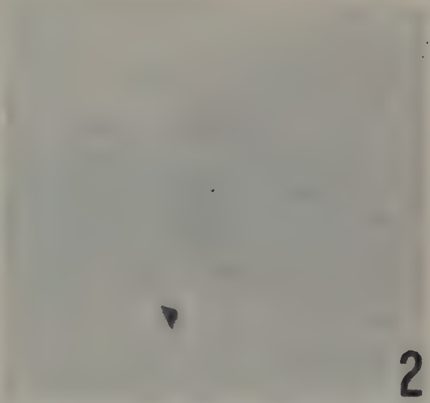

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FIGURE 1. Pitting disease on ripe Cavendish bananas; pits range in size from 1 mm to 7 mm in diameter.

FIGURE 2. Conidia of *Piricularia grisea* (approx. X 307.5).

infected tissue readily yielded the fungus *P. grisea.*

Temperature Studies: Circular plugs, 4 mm in diameter, of *P. grisea* mycelium grown on V-8 juice agar (2) were aseptically transferred to Ryan growth tubes (3) half filled with V-8 juice agar. Thrice weekly measurements were made to determine growth rates at 55°, 63°, 75°, 83°, and 89° F in three tubes at each temperature. Measurements after 21 days yielded the following mean linear growth of the fungus in the dark: 55° - 23.7 mm; 63° - 36.3 mm; 75° - 71.0 mm; 83° - 70.3 mm; 89° - 70.7 mm.

Comparison with an isolate of *P. grisea* from St. Augustine grass[2] grown under identical conditions yielded the following results after 21 days: 55° - 13.3 mm; 63° - 21.3 mm; 75° - 43.0 mm; 83° - 49.7 mm; 89° - 54.7 mm.

Obviously these two isolates differ in their temperature requirements. At temperatures studied, the banana isolate grew equally well over a wide range of temperatures of from 75° to 89° F, while the isolate from St. Augustine grass required a high temperature of 89° for maximum growth. The banana isolate, at each temperature studied, grew at a faster rate than did the isolate from St. Augustine grass.

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[2] Kindly furnished by Dr. J. H. Owen, University of Florida, Gainesville, Florida.

EVIDENCE AGAINST SEED TRANSMISSION OF GREEN RING MOTTLE VIRUS IN MAZZARD CHERRY SEEDLINGS[1]

R. M. Gilmer and K. D. Brase

Summary

The possibility of transmission of green ring mottle virus through seeds of infected mazzard mother trees was investigated. A random sample of 168 mazzard seedlings, open-pollinated progenies of two trees infected with green ring mottle virus, was indexed by bud-inoculation to Kwanzan or Shirofugen flowering cherry indicators. No evidence of seed transmission of the virus was obtained.

Recent reports (1, 2, 3) have indicated that symptomless infections with green ring mottle virus (GRMV) are common in sweet cherry (Prunus avium). The occurrence of GRMV in many different clones of sweet and mazzard cherry and of sour and Duke cherry suggested that infection might have been contracted from infected seedling rootstocks. The most likely avenue of infection of seedling rootstocks appeared to be through seed-transmission of the virus. The possibility of transmission of GRMV through seeds of mazzard cherry (P. avium), a common rootstock for cherries, was therefore investigated.

Seeds of two mazzard seed-source trees (T-33 and T-57), infected with GRMV but free of sour cherry yellows and necrotic ring spot viruses, were planted in the fall of 1959. In August 1960, 56 yearling seedlings, open-pollinated progeny of T-57, were selected at random from the nursery row and were indexed by inserting four buds from each seedling into Shirofugen indicators (P. serrulata) in the field. As controls, seven isolates of GRMV from sweet cherry were bud-inoculated to Shirofugen indicators. Two of these control inoculations were from the infected mazzard seed parents, T-33 and T-57.

Severe necrosis of leaf veins and marked curling and epinasty of the foliage developed in 1961 on six of the seven Shirofugen indicators inoculated with GRMV as controls. Initial symptoms were evident within 2 weeks after budbreak and were very pronounced by early June. The remaining GRMV control isolate was contaminated with necrotic ring spot virus, which killed the inoculated branches of the Shirofugen indicator before any symptoms of GRMV infection became apparent. None of the Shirofugen indicators bud-inoculated with buds of the T-57 seedlings developed veinal necrosis or epinasty, and all remained completely free from symptoms during the entire growing season.

In February 1961, before the results of the field experiment were known, a random sample of 112 seedlings, open-pollinated progeny of tree T-33, were indexed in the greenhouse. Individual rooted cuttings of Kwanzan flowering cherry (P. serrulata) were budded with two buds from a T-33 mazzard seedling just as the cuttings were breaking dormancy. Four GRMV isolates, again including those from the parent trees T-33 and T-57, were included in the experiment as controls. Greenhouse temperatures were maintained at 70° to 75° F (days) and 50° to 55° (nights) until late May, since prior experience with GRMV indexing with Kwanzan indicators had shown partial masking of symptoms by high temperatures.

Veinal necrosis and mild epinasty developed in each of the eight Kwanzan cuttings inoculated with GRMV as controls, including those inoculated from the parent trees T-33 and T-57. Symptoms were evident 6 to 11 weeks after inoculation, but were much milder than those that developed in the field on Shirofugen indicators inoculated with the same isolates. None of the Kwanzan cuttings bud-inoculated with buds of T-33 mazzard seedlings developed any foliage abnormalities.

These data demonstrate that GRMV is not seed-transmitted to mazzard seedlings from an infected maternal parent. The number of seedlings indexed seems adequate to have revealed even fairly low levels of seed transmission of GRMV had it occurred.

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PREVALENCE OF THIELAVIOPSIS BASICOLA IN CALIFORNIA CITRUS SOILS

Peter H. Tsao[1]

Summary

A sample-survey of the major citrus-growing California counties revealed that Thielaviopsis basicola was present in soils of more than half of the 126 orchards sampled. The fungus was more prevalent in the following southern California counties: Orange, San Bernardino, Riverside, Los Angeles, Santa Barbara, and Ventura, and was less widely distributed in San Diego, Imperial, and the three central California counties: Fresno, Kern, and Tulare.

Thielaviopsis basicola (Berk. & Br.) Ferr., a root pathogen with a wide host range, was known to be present in citrus soils (2) and was found to cause damage to citrus seedlings (3, 5, 6, 7). During the course of the investigation on this fungus as a citrus root pathogen, and since 1958, soil samples from citrus orchards selected at random in 11 major citrus-growing counties of California have been examined for the presence of this soil pathogen. T. basicola was found widely distributed in citrus soils, particularly in most of the southern California counties.

MATERIALS AND METHODS

All 11 major citrus-growing counties of California and a total of 126 citrus orchards were included in this brief sample-survey (Table 1). In most cases, two to eight soil samples containing fragmentary roots were taken from each orchard, at a depth of between 2 and 12 inches. Many of the samples were kindly provided by various farm advisors in these counties[2].

Table 1. Recovery of Thielaviopsis basicola from citrus soils in 11 major citrus-growing counties of California.

County	Number of citrus orchards: sampled	Number of citrus orchards: from which T. basicola was isolated	% recovery
Orange	10	9	90.0
San Bernardino	14	12	85.7
Riverside[a]	30	21	70.0
Los Angeles	6	4	66.7
Santa Barbara	12	8	66.7
Ventura	14	9	64.3
San Diego	12	3	25.0
Fresno	10	2	20.0
Kern	11	1	9.1
Tulare	4	0	0
Imperial	3	0	0
Total	126	69	54.8

[a] T. basicola was recovered from soils of 20 of 26 orchards sampled in western Riverside County, but from only 1 of 4 orchards sampled in Coachella Valley, which has a hot desert climate similar to that of Imperial County.

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[2] Appreciation is expressed to the following persons for providing some of the soil samples included in this study: M. H. Gerdts, G. E. Goodall, K. W. Hench, B. W. Lee, M. P. Miller, W. C. Morgan, K. W. Opitz, J. E. Pehrson, R. E. Puffer, and D. O. Rosedale.

A modification of Yarwood's carrot-disk technique (8) was used for recovering T. basicola from soils. Carrot disks were cut and briefly soaked in deionized or distilled water. Eight to ten disks were then placed in a covered, 16-oz paper cup containing approximately 100 cc of the soil sample. The cup was rotated to provide a thorough coverage of soil particles on the wet surfaces of the carrot disks. Three replicate cups were used for each soil sample, and a minimum of three cups containing carrot disks and an autoclaved soil was used as a control in each experiment. After a suitable incubation period, usually at 25°C for 3 to 4 days, the soil was washed away from the disks under the tap (deionized water), and the disks were further incubated in the washed, moist cup for an additional period -- up to 5 days after washing. Production of endoconidia and chlamydospores by the fungus, when present, usually began on the cut surfaces of the carrot disks 1 to 2 days after washing. The characteristic color and appear ance of the sporulating fungus made it easily recognizable macroscopically. Whenever necessary, subsequent identification of the fungus was confirmed by microscopic examination or culturing. A positive identification of the fungus in any replicate cup of a soil sample was considered evidence for the presence of the fungus in the sample.

The "carrot-half" technique (5, 7) was used for isolating T. basicola from root tissues. Ten to 30 1-cm long root pieces were used for each soil sample.

RESULTS

T. basicola was present in soils of 54.8% of the orchards surveyed (Table 1). The fungus was especially prevalent in six of the eight southern California counties: Orange, San Bernardino, Riverside, Los Angeles, Santa Barbara, and Ventura counties, which had as of 1960 a combined citrus acreage constituting about 70% of the total State citrus acreage (1). Recovery of T. basicola from the orchards surveyed in these counties ranged from 64.3% to 90.0% (Table 1). The fungus appeared to be less widely distributed (recoveries 0 to 25%) in San Diego and Imperial counties and the three counties in the Central Valley: Fresno, Kern, and Tulare.

The fragmentary fibrous roots in the soil samples were examined at random under a dissecting microscope. Typical lesions of T. basicola (7) were observed on roots from most of the infested orchards. Isolation of T. basicola from random root samples of 40 of the infested orchards yielded a recovery of 42.5%.

DISCUSSION

Martin and Ervin (2) isolated T. basicola from 8 of 60 orchards by the dilution-plate method using the peptone dextrose rose bengal-streptomycin medium. The higher percentage recovery of T. basicola in the present study (from 69 of 126 orchards sampled) indicated that the carrot-disk method was more sensitive than the dilution-plate method for detecting the presence of this fungus in the soil. The results of a comparative study of the two methods (unpublished data) have supported this view.

Despite the initial low percentage recovery obtained by Martin and Ervin, T. basicola was likely present in most of the soils used in their study. They reported that the recovery of the fungus from the 60 soils rose from the initial 8 soils to 11 soils after one cropping to orange seedlings in the greenhouse, and to 54 soils after 2 croppings (2). In view of these results, it is reasonable to assume that T. basicola probably has a wider distribution in California citrus orchards than the figures in the present study indicate. Furthermore, most of the soil samples used in the present study were collected during the summer months when high temperatures prevail in most of the citrus-growing regions of California. As the pathogenesis of T. basicola on citrus roots is enhanced by low soil temperatures and reduced by high soil temperatures (6), the percentage recovery of this fungus might have been higher in cooler months.

The carrot-disk technique as employed in this study revealed only a presence or absence of T. basicola in the soils tested. A quantitative "percent-coverage" method (4), also employing carrot disks, was developed recently and is useful in comparing the degrees of Thielaviopsis infestation in natural soils. This method will allow a more detailed study of the prevalence and distribution of this soil fungus.

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STUDIES ON EPIDEMIOLOGY OF BACTERIAL ANGULAR LEAFSPOT ON STRAWBERRY[1]

B. W. Kennedy and T. H. King[2]

Summary

The bacterial pathogen causing angular leafspot on strawberry survived for at least 10 years in strawberry leaves stored in a plant press in the laboratory. It also survived in leaves buried in field soil or suspended in the air above ground in Minnesota from one crop season to the next. The alpine strain of Fragaria vesca is highly resistant to angular leafspot, while the commercial varieties Robinson, Sparkle, and Trumpeter are relatively susceptible. The disease is favored by high humidity, moderate temperature, and rank growth of the plant.

Angular leafspot of strawberry occurs throughout the growing area in Minnesota. Probably widespread in this State for many years, the disease has undoubtedly been confused with fungus leafspots and heretofore escaped detection. As lesions age, it is increasingly more difficult to distinguish between the angular leafspot symptom and "common leafspot" caused by Mycosphaerella fragariae Lindau and "scorch" caused by Diplocarpon earliana Wolf. In the early stages, however, angular leafspot is clearly distinguished by small, water-soaked angular lesions which bear a milky slime when wet. Linford (5) described a similar disease in Utah in 1927, and implicated bacteria as the probable cause; he did not, however, prove a causal relationship between bacteria and the disease.

The present study was concerned with survival of the pathogen, varietal susceptibility and environmental factors influencing infection. Description of the disease, as well as the pathogenicity, host range and identity of the causal bacterium, is incorporated into other reports (2, 3, 4).

Survival of Inoculum: Strawberry leaves infected with bacterial angular leafspot collected from the Minnesota Fruit Breeding Farm, Excelsior, Minnesota, in 1949 and stored in a plant press in the laboratory were found to contain bacteria capable of causing angular leafspot in 1959. Water extracts from diseased leaves collected from the field in May 1959 and stored dry in an open box in the laboratory induced disease on leaves of healthy strawberry plants growing in the greenhouse in November 1961, indicating survival for at least 2 1/2 years under these conditions.

Therefore, a study was made to determine the effect of different storage conditions on the survival of the organism. In October 1959, infected leaves on the plant, infected leaves removed and placed in small nylon bags, and juice from infected leaves were treated as follows:

1. A small nylon bag of infected dry leaves suspended in the air outside.
2. A bag of infected leaves suspended inside a greenhouse maintained at about 18° C.
3. Infected leaves buried in 4-inch pots of field soil stored outside.
4. Infected leaves buried in 4-inch pots of field soil stored in the greenhouse.
5. Infected plants transplanted into soil in a field not previously cropped to strawberries.
6. Leaf juice from infected leaves injected into pots of soil stored in the greenhouse.
7. Leaf juice from infected leaves injected into root zone of potted strawberry plants growing in the greenhouse.

At periodic intervals a sample was recovered from each treatment except No. 5, suspended in water, and injected into leaves of healthy strawberry plants growing in the greenhouse to determine the viability of the pathogen.

The pathogen in extracted leaf juice alone could not be isolated from fallow soil (treatment 6) nor from soil and living strawberry roots (treatment 7) after 1 month. Leaves buried in soil in the greenhouse (treatment 4) did not yield the angular leafspot pathogen after 3 1/2 months. Infected leaves suspended in air, either outside or inside, and leaves buried outside yielded the pathogen on April 4, 1960, a time when plants in the field had begun to break dormancy. Soon after, symptoms were appearing on plants in the field (treatment 5), indicating that the organism had survived the winter in leaves and was able to cause infection on the new leaves as they appeared in the spring. These studies indicate that the causal organism can survive in dry leaves at 18° C or leaves buried in or above soil in the field, long enough to bridge the gap from season to season. The problem of overwintering of the pathogen would not appear to be a limiting one in Minnesota.

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Varietal Susceptibility: In 1959, observations on the severity of angular leafspot on 13 varieties of strawberries growing in the field indicated little difference in susceptibility. In the 1960 season, 20 varieties were planted in the field and 64 varieties were planted in the greenhouse for studies of susceptibility. In the greenhouse studies, one to six young plants were obtained from field plantings and placed in 4-inch clay pots where frequent watering kept humidity high until the plants became established. Approximately 2 months after transplanting, all plants were inoculated by wetting the undersurfaces of leaves by atomizing with a suspension of the bacterial angular leafspot pathogen. The bacteria used as inoculum in this test were contained in aqueous extracts taken from strawberry leaves infected with the angular leafspot disease (10 moderately infected leaves macerated and the extract diluted with 100 ml of water). Plants were kept in a moist chamber held at 18° to 21° C for a 24-hour period both before and after inoculation.

Fourteen days after inoculation, records were made on disease severity by rating two or three of the most severely affected leaves on each plant. Each rating consisted of a fraction in which the numerator, a number from 0 to 4, served to estimate the number of infection loci per leaf and was based entirely upon a relative comparison among reactions throughout the group of plants under test. A 0 rating indicated no infection while 4 represented the maximum number of infection loci. The denominator, also a number from 0 to 4, was based upon the size of individual lesions. The approximate diameter of lesions, as represented by numbers in the denominator, were as follows: Lesions given a rating of 1 covered an area approximately 0.5-1.0 mm in diameter; a 2 rating, 1.0-1.5 mm; a 3 rating, 1.5-2.0 mm; and 4, more than 2 mm in diameter with abundant coalescing of lesions and copious production of bacterial exudate. Thus, in each reading, the prevalence and size of infection loci on the leaf could be depicted.

Of the 64 varieties tested, the alpine strain of Fragaria vesca and Minnesota 1716 both rated 1/1, a value lower in the disease severity scale than any of the others (Table 1). Many gradations in disease severity were evident among the other 62 varieties, the Ambrosia variety being the most severely attacked.

Young greenhouse-grown plants representing 20 different varieties were transplanted to the field in April 1960 in randomized block design with three replications. A replicate consisted of two plants of each variety grown 1 foot apart while each pair of plants was 3 feet from the other plant pairs in the same row. Rows were 4 1/2 feet apart.

When plants had three to six trifoliate leaves at or near maximum size, they were inoculated by forcing bacterial suspensions into the leaves with the aid of a motor driven sprayer operating at 200 pounds' pressure. The spray stream of inoculum was applied at an angle to the plants so as to force leaves over, top side down, on the ground. By holding the spray nozzle approximately 5 or 6 feet away, leaves became visibly "water-soaked" within a few seconds. The inoculum used in the sprayer was derived from 1000 ml of a 6-day-old nutrient broth culture of the pathogen mixed with 100 liters of water. Inoculation was made when weather was cloudy and humid, immediately following a rain and readings of disease severity (2 to 4 of the most severely infected leaves on each plant) were made 15 days after inoculation.

Under these conditions, F. vesca did not develop symptoms of the disease. The varieties Robinson, Sparkle, and Trumpeter were more severely infected than any of the remaining 17 varieties (each of the three were rated 4/4). While Ambrosia was the only variety with a severity of 4/4 in the greenhouse experiment, it was less severely diseased (3/3) in the field. Unfortunately, Minnesota 1716, which showed resistance comparable to F. vesca in the greenhouse, was not included in the field experiment. No evaluation of its performance under these conditions is available.

Effect of Temperature and Humidity on Infection: Since the disease appeared early in the season under natural conditions and tended to diminish with the onset of hot weather, a study of temperature-moisture relations was made. In some experiments, plants were atomized with bacterial suspensions (aqueous extracts from diseased leaves) with sufficient force to thoroughly wet the undersurfaces of leaves but not produce water-soaking. In others, enough force was applied to produce visible water-soaking over approximately half the leaf area. Six plants were inoculated and incubated in each of four chambers maintained at 18°, 22°, 26° and 30° C; three of the plants were covered with plastic bags immediately after inoculation. The plastic bags were left on the plants for 4 days, then removed, but put in place again for 24 hours at weekly intervals. Plants were sprayed with water each time before covering.

Table 1. Severity of bacterial angular leafspot on various strawberry varieties grown in a greenhouse maintained at 20° to 28° C.

Variety	Disease severity[a]	Variety	Disease severity[a]
Alaska seedling	2/3	Minnesota 1788	3/3
Ambrosia	4/4	Minnesota 1839	3/4
Anchorage	2/4	Minnesota 1890	3/4
Armore	3/3	Northwest	3/3
Arrowhead	1/2	Orland	3/4
Badger x Catskill	1/4	Ozark Beauty	2/3
Beaver	2/3	Palmer	3/3
Blaze	2/2	Paymaster	3/3
Brilliant	3/3	Pocahontas	2/3
British Sovereign	2/2	Red Coat	3/2
Catskill	1/4	Red Glow	3/3
Cavalier	3/3	Red Rich	2/3
Doland	1/2	Red Star	2/3
Earlimore	1/3	Robinson	3/3
Empire	2/1	Senator Dunlap	3/3
Erie	2/3	Sioux	1/4
Eureka	1/2	Sitka	3/3
Fairfax	2/3	Sparkle	3/3
Fairland	2/4	Steelmaster	2/4
Fairpeak	3/4	Strafford	3/3
Great Bay	3/2	Superfection	3/3
Grenadier	1/4	Temple	3/4
Haines	1/4	Tennessee Beauty	3/3
Iowa	2/2	Tennessee Shipper	2/3
Jersey Bell	3/3	Trumpeter	3/4
Jumbo	3/3	Vermillion	1/3
Maine	2/2	Vineland	3/4
Massey	1/2	Wild Flavor	3/2
Mastodon	2/4	Wisconsin 214	1/4
Merrimack	3/3	Zeckcellen	3/2
Midland	3/2		
Minnesota 1716	1/1	*F. vesca* (alpine)	1/1
Minnesota 1728	3/3		

[a] Numerator represents relative number of infection loci. Denominator represents relative size of infection loci.

Disease readings were made by counting lesions 1, 2, 3 and 4 weeks after inoculation, records being made on the time required for infection to become evident, percentage of leaflet area infected and percentage of leaflet area necrotic.

At the end of 4 weeks, average numbers of lesions per trifoliate leaf on the three most severely infected trifoliate leaves of each plant were markedly reduced at 26° and 30° C regardless of humidity conditions. At 18° there was a 47-fold increase in numbers of lesions that formed on covered plants as compared with uncovered plants at the same temperature. Severity of disease was greatly increased by water-soaking immediately before inoculation. At 26° the disease caused severe necrosis in 4 weeks on every leaflet inoculated by the water-soaking method. Many of the leaves were completely killed and dropped from the plant. Leaves 4 to 8 weeks old were more susceptible than younger or older leaves irrespective of the method of inoculation.

Effect of Host Nutrition on Infection: To determine if there was an association between disease severity and plant nutrition, plants were grown in sand culture watered with solutions containing a complement of essential microelements and balanced, high and low concentrations of nitrogen, phosphorus, and potassium. These solutions were prepared similarly to those used by Gallegly and Walker in a study of the bacterial wilt disease of tomato (1).

Plants watered with solutions low in nitrogen became stunted and the leaves chlorotic, whereas leaves on plants watered with insufficient quantities of phosphorus or potassium remained small, became dark green in color and finally turned reddish-brown and died prematurely. These deficiency symptoms were evident after the fourth week. Little or no differences were noted in growth habit between the series of plants receiving complete nutrient solutions and the plants receiving high amounts of N, P, or K.

Healthy and vigorously growing leaves (on plants receiving high or balanced amounts of NPK) were consistently more severely attacked than plants in containers receiving solutions where N, P, or K was supplied in deficient amounts. Four weeks after inoculation, plants receiving balanced NPK, high N, high P, and high K had 15, 16, 20, and 19% of their total leaf areas necrotic, respectively, whereas those plants receiving low N, low P and low K had 3, 1, and 0% of their total leaf area necrotic.

DISCUSSION

In general, the bacterial angular leafspot disease of strawberry has many similarities in symptomatology and epidemiology to other bacterial leafspot diseases in other crops. Severity is correlated with high humidity, water-soaking of the host tissue, and rank growth of the plant. The pathogen is capable of surviving in dead plant material from one season to the next.

Many factors contribute to the successful establishment and spread of the angular leafspot disease in Minnesota. The fact that the causal organism can survive for long periods in leaves stored under a variety of conditions can enable overwintering in strategic areas for infection to occur the following season. Since varietal susceptibility is rather general and since moderate temperatures along with adequate and timely precipitation usually characterizes the growing season, spread and development of the disease would appear to be favored to some extent in most years. At any rate, conditions brought about by sprinkler irrigation greatly enhance development and spread of the disease. From experimental data obtained in the greenhouse, high humidity appears to be especially important at temperatures near 20° C.

Although some species of *Fragaria*, especially *F. vesca*, have shown a high degree of resistance, the factors responsible for resistance have not been investigated thoroughly. Macroelement deficiency (nitrogen, phosphorus, or potassium) can cause plants to become unhealthy and in turn become more highly resistant. Young and old leaves appear to be less subject to infection than healthy leaves of maximum size.

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A STUDY OF SYMPTOMS AND CONTROL OF PHOMOPSIS JUNIPEROVORA ON ARIZONA CYPRESS[1]

A. T. McDaniel and C. L. Wilson[2]

INTRODUCTION

The advent of Arizona cypress, Cupressus arizonica, as a cultivated Christmas tree has focused attention on disease control.

Phomopsis juniperovora Hahn (1) causes a needle blight of Arizona cypress which may result in death or loss of form and vigor of the tree. Heavy infections of P. juniperovora were encountered in a planting on the University farm at Fayetteville in 1961. Three fungicides were tested to determine their effectiveness in controlling this disease.

SYMPTOMS

Some confusion of the symptoms of P. juniperovora and Cercospora thujina appears to exist. Thompson and Murray (3) report symptoms of C. thujina to be much the same as those caused by P. juniperovora. They report that "Trees exhibit a reddish-brown dying back from the tips of branches." Wilson (4), however, reports "The disease (C. thujina) starts in the lower inner branches and progresses upward and outward." Hodges (2) reported of the disease on eastern red cedar, "The distinguishing symptom of the disease was a browning of the needles on the lowest branches adjacent to the stem. Infection gradually progressed upward and outward until only branch tips remained green on severely affected plants." It has been observed here that symptoms of the two diseases are distinct. P. juniperovora symptoms are necrosis of tips of laterals at the base of the tree, with necrosis progressing inward and upward and sporulation occurring in ashen gray areas near the bases of laterals (Fig. 1). C. thujina symptoms are necrosis of needles near the stem at the base of the tree, with necrosis progressing outward and upward and sporulation usually occurring on the proximal side of needles (4).

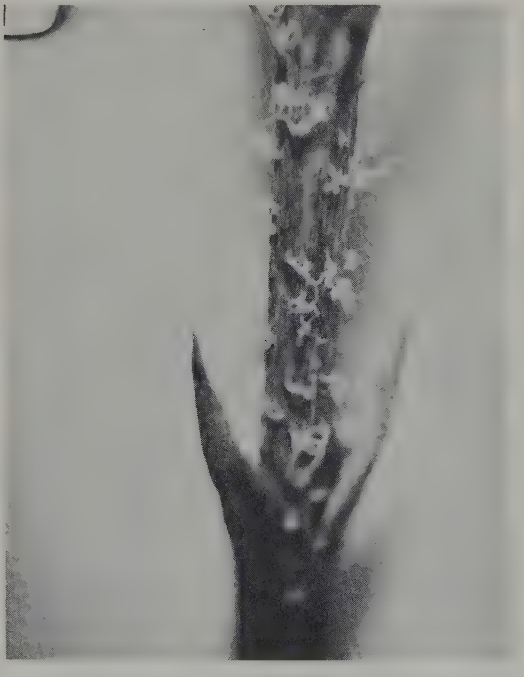

FIGURE 1. Cirri of Phomopsis juniperovora on Arizona cypress.

METHODS AND MATERIALS

Six hundred 1-year seedlings (1-0) were planted in March 1961. Symptoms of P. juniperovora occurred during a wet period in July 1961. Four treatments consisting of check (no treatment), Acti-dione RZ (2 oz. in 15 gallons water), maneb (Manzate, 4 oz. in 15 gallons water) and Bordeaux (4-4-50) with six replications of 25 trees each, were used in a randomized complete block design. The trees were rated prior to the first fungicide application according to severity of infection with 1) no infection, 2) lower one-third of tree infected, 3) lower two-thirds of tree infected, and 4) two-thirds to complete infection. A second rating of trees as to disease incidence was made September 19, 1961. Owing to differences in severity of infections in initial readings, data were adjusted to a common base by covariance.

Four applications of the fungicides were made at 14-day intervals: July 26, August 10, August 18 and September 1, 1961. The application on August 18 was made earlier than scheduled because of rain on August 15 and 16.

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[1] Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

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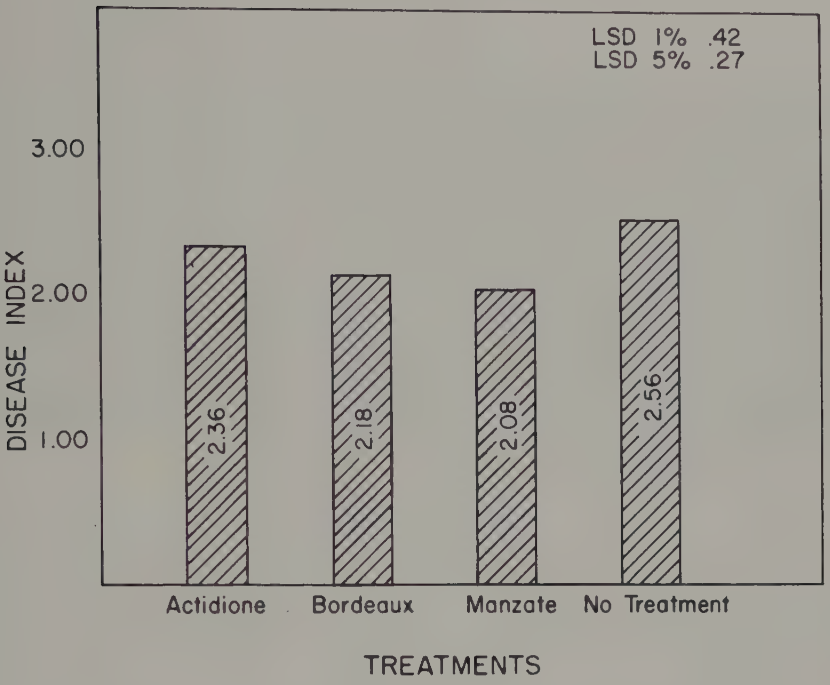
FIGURE 2.

* Average Disease Index as Affected by Treatments.



* These data were adjusted by covariance due to differences in initial infections.

RESULTS

Maneb and Bordeaux gave control, with maneb being superior to Bordeaux. Acti-dione gave no control over the check. Maneb was superior to the check at the 1% level and superior to Acti-dione at the 5% level. Bordeaux was superior to the check at the 5% level (Fig. 2).

DISCUSSION

This is a preliminary report and should not be taken as a recommendation for control of _P. juniperovora_ on Arizona cypress. It shows both maneb and Bordeaux to be active against this disease. Further work must be done to determine rates and time of application.

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AN EFFECTIVE PROGRAM FOR THE CONTROL OF BANANA MOSAIC[1]

A. V. Adam[2]

Summary

The occurrence of a major outbreak of banana mosaic in Central America is reported, the symptoms on Giant Cavendish bananas described and an effective control program discussed. This program involves elimination of plantains from the vicinity of banana plantations, regular periodic inspections of all banana farms, spraying areas of infection with an insecticide, eradicating the infected mat and 80 adjoining mats, spraying the area of eradication with an insecticide, spraying the 25 acres surrounding the eradicated area twice again at 20-day intervals, preventing regrowth of banana plants in the area of eradication, and replanting 6 months after eradication. This program reduced the incidence of banana mosaic in a 14,000-acre plantation in Honduras from an average of 21.15 infected banana mats per 1000 acres in 1956 to 0.12 mats in 1960 and, in conjunction with careful seed selection and treatment, has prevented the transmission of banana mosaic to any of the new farms established since the adoption of the inspection and eradication program. As far as can be ascertained, this is the only instance where a major outbreak of banana mosaic has been brought under practical control in a commercial plantation.

INTRODUCTION

In December 1955 an abnormal condition was observed on a number of Giant Cavendish banana plants in the Aguan River Valley of Honduras. A detailed inspection of the area revealed at least 175 mats showing typical symptoms of this condition in an area of 136 acres. This disease condition was identified as banana mosaic by Dr. C. W. Wardlaw of the University of Manchester, England, and by others, on the basis of symptom expression in the field.

The disease seems to have originated in Naranjo farm, which is one of the oldest existing banana farms in the Aguan River Valley and where the first recognized major outbreak took place. This farm, unfortunately, had been used as the major source of seed for a large number of newer farms in Honduras. Since December 1955 additional widely scattered cases have been located in the Aguan Valley. A considerable number of diseased plants were also encountered in certain Giant Cavendish farms on the north coast of Honduras between the towns of La Ceiba and Tela (Table 2. Ceibita, Carmelina, San Juan). As far as can be ascertained this is the first reported incidence of banana mosaic in this area of Honduras.

OCCURRENCE AND SYMPTOMATOLOGY

Banana mosaic has been found in various parts of the world (1, 2, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). Waite (11) recently reported that banana mosaic in Central America is caused by the cucumber mosaic virus. This was also indicated in 1934 by Wellman (16) and later by others (4, 6, 10). The theory has been advanced that several strains of cucumber mosaic may be responsible for this disease (11). Apparently symptom expression differs to a certain extent according to banana variety and sometimes according to location.

Infected Giant Cavendish plants in the Aguan Valley and on the north coast of Honduras exhibit the following symptoms:

1. Some rosetting of the leaves. However, the Cavendish banana is prone to develop this characteristic when growing under various unfavorable environmental conditions, which lessens the reliability of this symptom in identifying the disease. In field inspections, however, it is a most useful though non-specific symptom in that it can be seen from a distance and does not necessitate close examination of every plant.

2. Stunting and unthriftiness of severely infected plants. This is particularly true with plants that are relatively young when infected, whereas those infected somewhat later quite

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[1] Acknowledgment is extended to Dr. J. R. Warren, Mr. R. A. Gross and Mr. B. R. Hogge for their prominent role in initiating work in this project and to Dr. M. L. Karon, Director of Research, for his coordination of the entire program.

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often reach normal or near normal height and diameter.

3. Distortion and yellowish discoloration of the heart-leaf and to a lesser extent also of the two or three top leaves. Like rosetting, however, this symptom may be caused by factors other than mosaic infection.

4. Reddish-brown to dark-brown subepidermal discoloration within the mid-rib of the leaf. This symptom appears quite early and is most apparent at the base of the mid-rib when viewed from the lower or bottom side. Among the several external symptoms, this is one of the most reliable and specific ones; it can be seen from a distance and does not necessitate destruction of the plant to ensure reasonably positive identification of the disease.

5. Yellow mottling between the lateral veins on the leaves. This symptom is somewhat more difficult to observe, especially from a distance, and apparently develops after the mid-rib discoloration. It appears first as a series of tiny yellow dots that gradually elongate into short dashes that may join end to end to form long yellowish streaks extending from near the mid-rib to the border of the leaf blade. In very advanced stages these streaks or dashes change from yellow to light brown. Usually, not all the leaves on an infected plant show this symptom.

6. Internal discoloration of pseudostem tissue. A cross section cut through the pseudostem shows soaked, necrotic, randomly located, pockets of tissue which, early in the development of the disease, become yellow to reddish-brown to brown in color. When the leaf sheaths which constitute the pseudostem are peeled apart, the discolored areas are seen to extend lengthwise along the petioles. This is one of the key symptoms in the identification of the disease.

7. Visual symptom expression less likely to occur on small plants.

8. Distortion and malformation of fruit produced by severely infected plants. Such fruit is small in size and rarely reaches commercial grade.

9. Reddish-brown, necrotic, discoloration of vascular tissues in the true stem or fruit stalk.

TRANSMISSION

An attempt was made to transmit banana mosaic mechanically. Ten plants exhibiting typical disease symptoms were selected and used for inoculating 100 apparently healthy ones. Inoculation was made by repeated machete stabs from each diseased to ten healthy plants. An equal number of controls was maintained by stabbing healthy plants with a sterilized machete that previously had been introduced into other apparently healthy ones. None of the inoculated plants developed mosaic symptoms.

No attempt was made to determine the specific vector or vectors responsible for the transmission of the banana mosaic virus in Central America. Waite (11), however, working with pot cultures, reported successful transmission of the virus between Musa and Ensete by Aphis gossypii, Rhopalosiphum prunifoliae, R. maidis, Myzus persicae and Macrosiphum pisi. He achieved no transmission of the disease when Pentalonia nigronervosa, which feeds more regularly on Musa than the above-mentioned species, was reared on infected banana plants and transferred to healthy Gros Michel in pot tests. Preliminary field studies by the same worker have not been conclusive as to which aphid species are responsible for transmitting banana mosaic in the field.

METHODS OF ORGANIZATION

At the time of the original outbreak, a heavy infestation of aphids, the majority of which were Pentalonia nigronervosa Coq., was evident in the vicinity of the infected plants. Although there was no positive evidence that these particular insects were responsible for transmitting the virus, it seemed advisable to eliminate them from infected areas. An application of insecticide was followed by immediate and complete destruction of plants showing symptoms of the disease. Because plants growing in the immediate vicinity of infected mats could also be infected, even though they might not yet show visible symptoms, an attempt was made to destroy these possible reservoirs of infection.

In detail, the mosaic eradication program that has been used in all of Standard Fruit Company's plantations in Honduras since the original outbreak consists of the following operations: 1) Regular mat-by-mat inspection of each farm at the approximate rate of three times a year. 2) Spraying an area of 25 acres surrounding the diseased mat with nicotine sulfate (1 pint of 40%/100 gallons of water). Sufficient spray liquid is applied to ensure thorough coverage of the standing plants as well as the undergrowth (200 to 250 gal. /acre). The bases of mid-ribs, where the majority of aphids and other insects are likely to be found, are thoroughly wetted. 3) Digging out the infected plant and 80 potentially infected mats (0.47 acre) surrounding the diseased plant, cutting into small pieces and exposing to the sun to stimulate rapid decomposition. 4) Spraying chopped plants with malathion E (1 quart per 100 gal. /of water). 5) Spraying the 25 acres surrounding the eradicated area again with nicotine sulfate at 20- and 40-day intervals following eradication. 6) Making certain that no regrowth of banana plants occurs in the eradicated area until the date specified for replanting. 7) Replanting the area 6 months after eradication, unless new infections appear in the vicinity of the eradicated plant, in which case replanting is delayed for 6 additional months. 8) Eradication of various members of the plantain group (Macho or Horn) from the immediate vicinity of banana farms. This appeared advisable after a large number of plantains, which were widely distributed through the farms, were found to possess symptoms strikingly similar to banana mosaic on Giant Cavendish. However, there is no positive evidence that this virus can be carried from plantains to Giant Cavendish bananas or vice versa, and no attempt was made to cross-inoculate them.

RESULTS AND DISCUSSION

Tables 1 and 2 show the incidence of mosaic in the Standard Fruit Company's plantations in Honduras during the last 5 years. The most spectacular drop in mosaic incidence occurred in 1957, the year after the original outbreaks had been controlled. Also, incidence of the disease has consistently declined during the last 5 years, reaching an all time low in 1960 with only six cases in approximately 14,000 acres of Giant Cavendish bananas.

Table 3 suggests that there is a trend in disease incidence, or symptom expression, toward more infection during the latter part of the cool, rainy, winter season and early part of spring (January, February, March, April). In this connection it is interesting that incidental observations over the last 5 years indicate that the highest number of aphids in this area occurs in October, November and December.

Although the insect vector responsible for transmitting the virus is not known, it seems very likely that the insecticidal applications also eliminated the true vector or vectors.

By selecting banana seed from areas with no mosaic history, and by prior detailed inspection of seed bed areas followed by individual seed selection, it has been possible during the last five years to prevent banana mosaic from spreading into new farms.

Table 1. Mosaic incidence per year in approximately 14,000 acres of Giant Cavendish bananas.

Year	Total no. of cases treated	Total acres inspected[a]	Av. incidence per 1000 acres inspected
1956	342	16,170	21.15
1957	51	47,462	1.07
1958	17	42,391	0.40
1959	15	39,878	0.37
1960	6	48,460	0.12

[a] Represents a number of inspections of each farm per year.

Table 2. Trend of mosaic incidence in the ten most severely infected banana farms.

Year	Carmen (450 A)	Ceibita (650 A)	Copete (630 A)	Carmelina (600 A)	Cayo (1100 A)	Naranjo (1100 A)	Palo Verde (600 A)	Rosario (900 A)	San Juan (550 A)	Trojas (1000 A)	Totals
1956	7	20	12	14	0	179[b]	23	19	7	29	310
1957	0	2	2	2	9	18	1	5	0	0	39
1958	3	0	3	1	0	3	0	0	0	2	12
1959	0	1	1	0	0	3	0	0	0	1	6
1960	1	-[a]	1	-[a]	0	3	1	0	-[a]	0	6

[a] Farm abandoned.
[b] Site of original mosaic outbreak.

Table 3. Seasonal trends in mosaic incidence in Naranjo farm[a] (approximately 1100 acres).

Year	No. of cases per inspection												Total no. of cases
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
1956	-[b]	175[c]	4	-	-	-	-	-	-	-	-	0	179
1957	8	9	0	-	1	-	-	-	-	0	-	-	18
1958	-	-	-	3	-	-	-	0	-	-	0	-	3
1959	-	0	-	-	-	2	-	-	1	-	-	-	3
1960	1	-	-	1	-	-	-	0	-	1	-	-	3
Av. no. of cases per inspection	4.5	4.5	2.0	2.0	1.0	2.0	-	0.0	1.0	0.5	0.0	0.0	

[a] Site of original outbreak.
[b] Not inspected.
[c] Original outbreak - Not included in the average.

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ADVANTAGES OF DETERMINING THE TOLERANCE OF STRAWBERRIES BY GRAFTING WITH LEAVES INFECTED WITH A VIRUS COMPLEX[1]

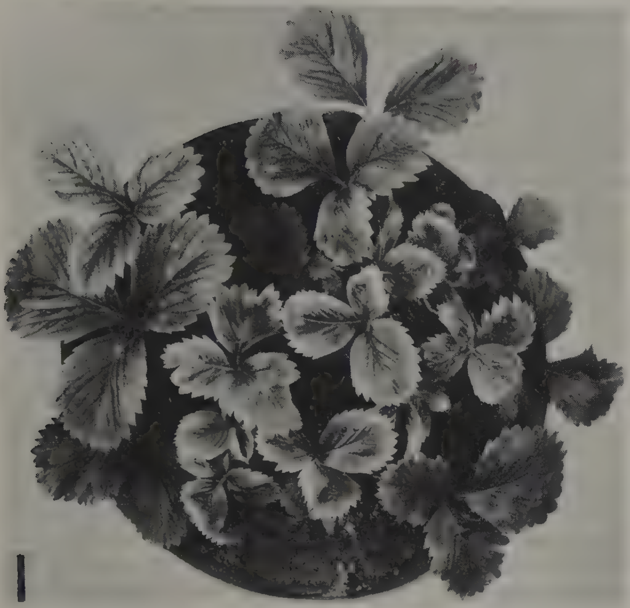
P. W. Miller[2]

In 1959, the author[3] described an improved method of testing the tolerance of named strawberry varieties and selected seedlings to virus infection. In the method outlined, the virus components were introduced singly into a plant by individual excised-leaf grafts, each bearing a virus entity. While this procedure is still considered to be the most enlightening, it is not very practical when many varieties or selected seedlings are being tested because it involves considerable time, large numbers of plants and a great deal of space.

Currently, one diseased Marshall clone containing the virus components usually prevalent in Oregon is being used as an inoculum, two excised-leaf grafts being made per plant[4]. The clone used contains the mild yellow edge, crinkle and mottle components and causes severe plant degeneration of the Marshall variety in a relatively short time (Fig. 1).

Virus symptoms usually appear much sooner when such a compound inoculum source is used and plant degeneration in sensitive varieties is more rapid in a shorter period than when the virus components are introduced singly. For example, on a variety or selected seedling which is very sensitive to the aforementioned virus complex, symptoms generally become visible in 3 to 5 weeks after inoculation and severe plant degeneration occurs in 6 to 8 weeks (Fig. 2).

The procedure of inoculating with a compound inoculum is considered to be more realistic because, in the Pacific Northwest, strawberry viruses generally exist and are spread as complexes composed of two or more viruses..



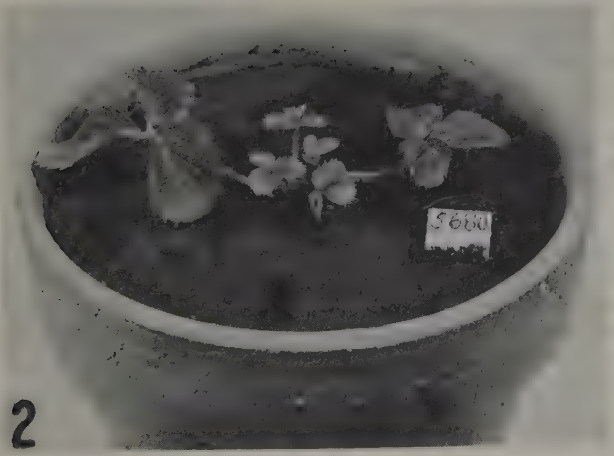


FIGURE 1. A plant of the Marshall variety naturally infected with a severe yellows virus complex composed of the mild yellow edge, mottle and crinkle virus components; used as a source of inoculum. (Photographs by H. H. Millsap) FIGURE 2. A plant of U. S. -Ore. strawberry selected seedling No. 2866 inoculated with the severe yellows virus complex shown in Figure 1 by excised-leaf grafts; photographed 70 days after grafts were made.

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OCCURRENCE OF CERTAIN PLANT DISEASES IN KENTUCKY IN 1961

E. M. Johnson, R. A. Chapman, Lawrence Henson, and W. D. Valleau

Low temperatures and high rainfall occurred throughout the State during the tobacco plant bed season, at setting time and well into June. After this, rainfall was rarely excessive and in some areas was low until well after tobacco cutting time in September.

TOBACCO

Bed Diseases: Wildfire (*Pseudomonas tabaci* (Wolf & Foster) Stevens) and angular leaf spot (*Pseudomonas angulata* (Fromme & Murray)) were present in a few beds but damage from either was rare. Wildfire was found on one farm in beds of wildfire-resistant Burley 21. An isolate from these beds was much more virulent than those from susceptible varieties and regularly caused typical wildfire on Burley 21 and *Nicotiana longiflora*.

Blue mold (*Peronospora tabacina* Adam) appeared in a few tobacco beds over most of the State during the last days of May and as late as June 26. The disease was so inconspicuous that most growers either were unaware of its presence or ignored it.

Anthracnose (*Colletotrichum destructivum* O'Gara) was present in a few beds in most areas of the State but caused little damage except in the southwestern counties. Even in this area the damage was not great enough to recommend the general use of chemicals for control. When beds were so constructed that there was no flooding from grass-legume sods, the disease was rarely present.

Field Diseases: Pythium soft rot (*Pythium* spp.) occurred rarely. In 1959 and 1960 some growers were forced to disk up and reset entire fields because of the destruction of so many plants by Pythium soft rot. In 1961 there were no reports of crops having to be reset because of Pythium soft rot.

Blue mold developed in some fields from spore showers. Generally, greater infection occurred near beds. A few circular necrotic spots up to a half inch in diameter developed on a few leaves of scattered plants. Sporulation in the field was light and damage was negligible. No infections were found in the field after mid-July. Blue mold destroyed small tobacco seedlings in a glass greenhouse in late August. The disease was still present and sporulating in this greenhouse on seedlings in flats and in small potted plants in late December. By January the disease had disappeared.

Anthracnose appeared to be less prevalent than last year and caused no damage.

Black shank (*Phytophthora parasitica* Dast. var. *nicotianae*) appeared no more prevalent than last year in spite of heavy rains early and flooding of black shank-free areas from infested fields. For many years following the appearance of black shank in the State in 1935, the disease has been confined for the most part to overflow creek and river bottoms. In the last few years, black shank has appeared in previously black shank-free areas in fields not subject to overflow. Burley 11A, 11B and 37 usually produce satisfactory crops on infested fields.

Frogeye (*Cercospora apii* Fres.) developed late and because of low rainfall caused little damage. Greenspot in cured tobacco was more prevalent than usual.

Wildfire continues to be of minor importance in burley because a high percentage of the crop is wildfire-resistant Burley 21. One field of Burley 21 set from a wildfire-infested bed showed appreciable damage from wildfire following a rainy period in July. Leaves halfway up the stalks often were covered with wildfire spots.

Angular leaf spot appears to be increasing in amount yearly. However, little damage occurred in most areas this year because rainfall was light during much of the growing season. Burley 21 and burleys with the wildfire resistance factor from 21 show less angular leaf spot than susceptible varieties.

Black root rot (*Thielaviopsis basicola* (Berk. & Br.) Ferr.) was present and reduced growth in many fields, especially in those used for tobacco for several years in succession. The black root rot-resistant varieties now grown do not have sufficient resistance to black root rot except in rotations.

Manganese toxicity was more prevalent than usual. It occurred throughout the State on soils of high acidity (pH 4.2-5.2). For some time following setting affected plants are chlorotic and grow slowly. Affected plants often develop 1/8- to 1/4-inch irregular-to-circular necrotic spots, usually on older chlorotic leaves. Analyses of manganese-toxicity-affected plants from different areas of the State showed a range of 2400 to 7400 ppm of manganese. Normal plants contain about 400 ppm. Manganese toxicity occurs more often in fields where tobacco is grown in continuous culture with heavy annual applications of commercial fertilizer. Soils in these fields tend to become very acid and gradually develop a very poor physical condition.

Organic matter toxicity occurred in greater amounts and persisted longer than in past years. This year, owing to heavy rains, organic matter either as animal manure or cover crops was turned under late. Decay was slow because of low temperatures. It is believed that the slow decay of organic matter results in the production of toxic material which destroys tobacco roots. In some fields in late June to mid-July there were areas where plants actually appeared smaller than at setting. The original roots of affected plants were dead, and often new roots that had developed were partially rotted. In some fields affected plants never attained sufficient size for cutting. Owing to the increase in the 1961 tobacco allotments, many farmers plowed sod strips adjacent to their continuous tobacco field. In all instances where organic matter toxicity was prevalent in the continuous tobacco area, the tobacco growing after sod in the adjacent strip was free from organic matter toxicity and was much larger than the adjacent continuous tobacco.

Chemical injury. Ten pounds per acre of Randox-T (chloro nn diallylaceto-amide 10%, trichlorobenzyl chloride 25%, inert ingredients 65%) were used sometime in early May as a row pre-emergence weed control treatment in a corn field. Six or more weeks later in July new leaves of tobacco, across a roadway from the treated corn, set after the corn was treated developed narrow, closely veined, very thick distorted leaves above leaves showing normal growth. Some affected tobacco leaves resembled those injured by 2,4-D, but for the most part the leaves were narrower. In some instances only a thickened midrib developed. Injury to the tobacco was greater where the row applicator turned in the tobacco field, but injury to the tobacco was not confined to these areas. Tomato and morning glory plants near the corn showed distortion. Burley plants with six leaves in 3-inch pots were placed for 12 hours under a bell jar with 10 grams of Randox-T in a Petri dish. All newly developing leaves and suckers showed the same type of distortion observed in the field plants injured by Randox-T. These plants were set in a ground bench in the greenhouse. Seven months following treatment, the plants continued to develop only narrow, thick, distorted leaves.

Magnesium deficiency or sand drown was not observed in tobacco in Kentucky until 1961. Typical symptoms of magnesium deficiency were observed in burley tobacco in a sandy soil area where tobacco had been grown for several years with heavy annual applications of commercial fertilizer. The soil had a pH of 5.9, was high in exchangeable potassium (768 pounds per acre), high in phosphoric acid (894 pounds per acre), and low in magnesium (189 pounds per acre).

CORN

Manganese toxicity occurred in corn under the same high soil acidity that induced the disorder in tobacco. Affected plants were reduced in size, and the upper leaves were chlorotic. Small necrotic spots were numerous on lower leaves. Affected plants contained 150 to 460 ppm of manganese, whereas normal plants contain about 50 ppm.

Pythium stalk rot of corn (Pythium sp.) occurred in one field in Ballard County.

ALFALFA

In some fields throughout the State, alfalfa was affected with a spotting of leaves, petioles and stems. The spots on the leaves were at first small, irregular and dark brown. Later these areas sometimes became zonate. On stems and petioles, the lesions were depressed brown spots. Stemphylium sp. developed in these spots usually after a night in a moist chamber. Whether Stemphylium was responsible for poor growth of alfalfa is not known.

FLOWERING CRAB APPLE

Apple scab (Venturia inaequalis (Cke.) Wint.) was present and often destructive on flowering crab. Unsprayed apples, and in some areas sprayed orchards, were injured greatly by apple scab. There appeared to be great differences in susceptibility among the varieties of flowering crab. Some varieties were almost defoliated, while other varieties in the same dooryard were little affected.

ELM

Dutch elm disease (Ceratocystis ulmi (Buis.) C. Moreau) was identified by isolation from one tree each in Barren, Jefferson and Fayette counties.

SYCAMORE

Anthracnose (Gnomonia veneta (Sacc. & Speg.) Kleb.) was more prevalent and injurious than usual on sycamore. Few trees escaped and many were almost defoliated. It was not unusual to see trees with branches intermingling where one was almost defoliated and the other little affected. Anthracnose appeared more destructive in trees along streams.

ASH

Anthracnose (Gloeosporium aridum Ell. & Ev.) was present and often destructive on ash. Some trees were defoliated. These produced a second crop of leaves which later were injured by anthracnose.

HOLLY

Specimens of Japanese holly (Ilex crenata) with a general unhealthy appearance were found to have many black roots. Thielaviopsis basicola was isolated from these black roots. The holly isolate appeared morphologically identical to tobacco isolates of this fungus. Whether infection with Thielaviopsis basicola was responsible for the poor growth of holly was not determined.

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REDUCTION OF SOIL POPULATIONS OF THE CITRUS NEMATODE BY THE ADDITION OF ORGANIC MATERIALS

R. Mankau and R. J. Minteer[1]

Abstract

Of eight organic materials added to soil infested with Tylenchulus semipenetrans, only steer manure failed to cause substantial reductions in numbers of larvae in 84 days. Castor pomace eliminated all citrus nematode larvae from the soil but apparently did not contain substances toxic to nematodes. Environmental factors associated with increased microbial activity following organic amendments appear to be unfavorable for survival of citrus nematode larvae in fallow soil.

INTRODUCTION

The possibility and desirability of using certain organic soil amendments for citrus culture prompted an investigation of the effect of available materials upon the primary nematode pest of citrus in California, Tylenchulus semipenetrans Cobb, 1913. In this preliminary study extravagant amounts of eight organic materials and an unamended control were compared with respect to survival of citrus nematode larvae in host-free soil. The results obtained gave a good indication of the relative effectiveness of the materials in reducing survival of citrus nematode larvae in fallow soil.

Several workers have examined the effect of addition of organic amendments to soil upon plant-parasitic nematodes. Linford, Yap and Oliveira (8) demonstrated reduction of a root-knot nematode (Meloidogyne sp.) population during decomposition of chopped pineapple plants, cane sugar and a coarse grass in soil. Feder (3) also observed reductions of nematodes after adding sugar to soil. Johnson (5) reported the reduction of severity of root-knot disease on tomato by 11 crop residues incorporated into soil and Lear (7) obtained significant reductions of three phytophagus nematode species with large quantities of castor pomace added to infested soil. Others have indicated that organic manuring suppresses the rate of infestation and reproduction of several Heterodera spp. (Duddington, et al. (1, 2), van der Laan (6), Oostenbrink (11), and Reinmuth (12)). Some of these investigations have involved the use of excessively large quantities of organic material and none of the practices have been generally adopted for control of plant-parasitic nematodes.

MATERIALS AND METHODS

Infested soil used in this test was obtained from an experimental plot planted to sweet orange seedlings at the Agricultural Experiment Station, Riverside, California. The soil contained approximately 3650 citrus nematode larvae per 100 cc when collected (Baermann funnel extraction). Fifty grams of each of the dry amendment materials listed in Table 1 and 20 ml of liquefied fish fertilizer were added to 2-liter aliquots of this moist soil, a rate equivalent to about 22.5 tons/acre for the dry amendments and about 2149 gal./acre for the liquid fertilizer. These materials were mixed thoroughly with the soil and then 100-cc portions were placed in 4-oz polypropylene plastic cups with tight-fitting lids. The soil remained moist during the test and additional water was not required. Four replicates per treatment were used.

After 84 days at 22° C, the contents of each cup were placed on a modified Baermann funnel and extracted for 48 hours. The nematodes obtained were counted with a Hawksley nematode counting slide (4) (Table 1).

Two additional studies also were made on the effect of the castor pomace on nematodes. In the first test, this material was added at rates of 2.5, 5.0, 10.0, 20.0 and 40.0g/liter soil. The second test attempted to determine whether the mammalian-toxic compound ricinine, present in castor pomace, affected citrus nematode larvae. Castor pomace, which had been extracted with hot acetone in a soxhlet extractor for 48 hours to remove ricinine, was added to at the rate of 25g of dry material/liter soil. Nontreated castor pomace also was mixed with soil. Each treatment was replicated five times and incubated for 30 days.

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Table 1. Effect of organic soil amendments on bacteria-feeding nematodes and on the survival of citrus nematode larvae.

Treatment	Number of citrus nemas	Adjusted mean	% reduction from control	Number of microphagus spp.	Final pH of soil
Nontreated	9795	97.67	--	380	7.7
Steer manure	7337	85.04	25.1	2510	7.9
Chicken manure	3915	61.47	60.1**	5205	7.4
Cotton waste	3075	54.70	68.7**	9915	7.8
Sugar beet pulp	2235	46.05	77.2**	13055	7.1
Alfalfa pellets	1780	39.77	81.9**	5910	6.9
Alfalfa hay	924	30.20	90.6**	4705	6.9
Liquefied fish	35	--	99.7**	320	6.0
Castor pomace	0	--	100.0**	43275	5.7

**Significant at 0.01 level, $S_{M.D.}$ = 10.63, L.S.D. of adjusted mean at 95% level = 26.36

RESULTS

Numbers of citrus nematode larvae were reduced by 50% or more in all amended soils except those that received steer manure. The castor pomace additive eliminated all of the citrus nematode larvae from the soil and the liquefied fish fertilizer almost all of the larvae. Alfalfa hay and ground alfalfa pellets also produced very substantial reductions in larvae. The increase in citrus nematode numbers in the control soil over the population level of the soil when collected probably resulted from the hatching of larvae from eggs in the soil, since small pieces of infested roots bearing egg masses occurred in the soil.

The treatments are arranged in Table 1 in descending order of percentage reduction of citrus nematodes. Reductions of over 25% are highly significant, based on a one-sided t test of square roots of the number of nematodes. With a reduction in citrus nematodes there was an increase in the number of microphagus nematodes in the various treatments, except that of the liquid fish treatment. Increases in microphagus species, which were almost entirely bacteria-consuming forms, reflect accelerated microbial activity in the soil. Some ammonia produced in the liquefied fish treatment, detected by its odor, may have caused the low numbers of nematodes of both types.

Since the castor pomace amendment proved to be very efficient in eliminating citrus nematode larvae, an additional test was conducted using a series of lower rates of that material in the same soil. Counts of citrus nematode larvae and microphagus types were made after 30 days' incubation (Fig. 1).

Bacteria-feeding nematodes increased in numbers with increased castor pomace additive and the numbers of citrus nematode larvae were decreased. The difference between the number of citrus nematode larvae in the 10g/liter treatment and the control is significant at the .05 level. No citrus nematode larvae survived the 20g and 40g/liter treatments.

Both the ricinine-free and non-extracted castor pomace killed all the citrus nematode larvae in the soil. Other nematode types increased greatly over controls in both castor pomace treatments. The differences in numbers of citrus nematodes between the treatments were not significant; however, significantly increased numbers of Aphelenchus avenae occurred in soil receiving the non-extracted additive. This may have been due to the removal of acetone soluble material which supported the growth of fungi upon which this nematode feeds. Small quantities of soil from each replicate of the three treatments were plated on weak corn meal agar. Three species of nematode-trapping fungi, Arthrobotrys arthrobotryoides, A. conoides and A. dactyloides, developed on plates from soil amended with castor pomace, while none occurred on plated control soil.

Observations on the presence of nematode-trapping fungi in the other experiments reported here were unsuccessful due to development of small enchytraeid worms in the plates which obscured any development of fungi on the agar. However, when some of the numerous bacteria-feeding nematodes from the castor pomace treatment were placed on water agar in Petri dishes, almost all of the nematodes in the agar were killed within a few days by the parasitic fungus, Harposporium anguillulae. It is doubtful that this fungus could have any effect upon citrus nematode larvae since its spores germinate in the gut of nematodes after being ingested -- the minute lumen of the stylet of citrus nematodes preclude the parasite, but it may control the buildup of microphagus nematodes.

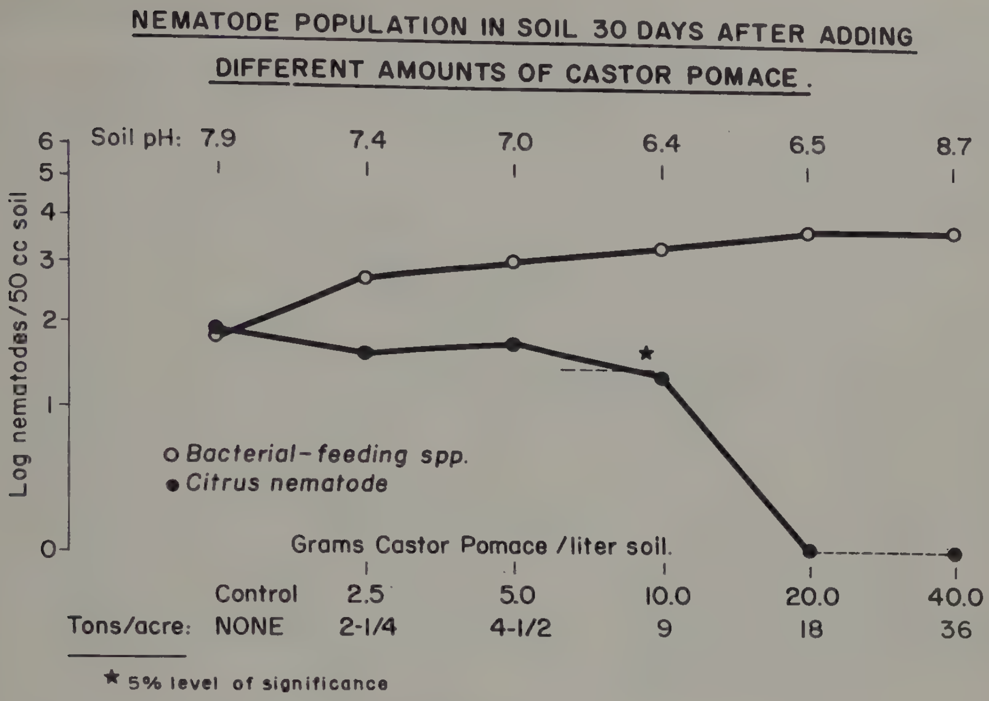

FIGURE 1.

Citrus nematode larvae obtained from infested citrus roots were exposed for 24 hours in small vials to 1:10, 1:100 and 1:1000 dilutions of substances recovered from the acetone refluxed through castor pomace, aqueous leachates of castor pomace, and also to distilled water. At the end of this period the vials were inverted in a small dish of distilled water and the viability of the larvae determined by counting those which passed through a fine mesh polymax nylon screen in the cap of the vial (10). None of the dilutions of either solution caused any significant reduction in larvae which passed through the screens.

Soil pH measurements were made on all replicates when they were sampled for nematodes and reduced citrus nematode larvae survival in all experiments could be correlated with an increase in soil acidity. Citrus nematode numbers decreased markedly in treatments in which amendments reduced pH below 7.0.

DISCUSSION

The reduction of a plant-parasitic nematode in infested soil by the addition of organic materials, some commonly used in agriculture, has significant and interesting applications in disease control. The large quantities of some amendments used in this study may be of limited practical value, but the influence of various materials on a nematode population in this study has important implications. The rapid elimination of citrus nematodes from infested soil mixed with castor pomace, a by-product of castor oil extraction, is very striking when compared with the other materials used. Lear (7) suggested small amounts of a material toxic to nematodes may be present in this material; however, the large increase in microphagus nematodes observed in this test when castor pomace was added to soil and the lack of any direct effect of extracts of castor pomace upon citrus nematode larvae in water appears to eliminate this possibility.

The reduced survival of citrus nematode appears to correlate well with an increase in microbial activity in the soil measured indirectly by numbers of bacteria-feeding nematodes present. In the alfalfa treatments, however, microphagus nematode numbers are somewhat lower than in some treatments in which citrus nematode reductions were not so great. Possibly fungi were more active in decomposing the alfalfa amendments than bacteria.

Toxic metabolic products from soil microorganisms or unfavorable environmental conditions created by microbial activity may be factors in the reduction of the citrus nematode. Further studies are in progress to explore these possibilities. Increased activity by natural enemies of nematodes may be partially responsible. Addition of organic materials appears to stimulate activity of predacious fungi in some cases (2, 8, 9). This was demonstrated in some tests with castor pomace in this study.

Additional experiments will examine the influence of organic amendments upon the citrus nematode in association with the host. However, information from this series of experiments has possible application in citrus replant situations where old citrus may be replaced by annual crops before replanting to citrus.

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OBSERVATIONS ON THE SUCCESSION OF FUNGI ON HARDWOOD PULPWOOD BOLTS

Alex L. Shigo[1]

Summary

Ceratocystis coerulescens was usually the first fungus to infect ends of hardwood bolts cut for pulpwood. The expanding mycelial patches were commonly overgrown by *Cephalosporium* sp., *Gliocladium roseum*, and *Trichothecium roseum*, which were responsible for changing the color of the ends from black to white.

OBSERVATIONS

A small quantity of all species of northern hardwoods (beech, sugar maple, red maple, paper birch, yellow birch, and American ash) are regularly cut for pulpwood on the U. S. Forest Service's Bartlett Experimental Forest at Bartlett, New Hampshire. Most of the cutting is done in the summer. The bolts are stacked along the roads until removed several weeks to a few months later.

The cut ends of many pulpwood bolts were examined periodically during the summer and fall of 1959, 1960, and 1961. The succession of fungi on the bolts cut each year was found to be the same. The changing colors and patterns on the cut surfaces were very obvious. Small dark spots, usually near the loosened bark, were seen on some bolts 2 weeks after they were cut. The spots enlarged quickly until most of the surface around the center portion of the bolts was covered. This was most obvious on beech, red maple, and paper birch. On many bolts, small white dots appeared in the centers of the expanding black patches (Fig. 1). The white dots enlarged until the black patches were covered and appeared appressed (Fig. 2). The ends of many bolts were cut off and taken to the laboratory for examination.

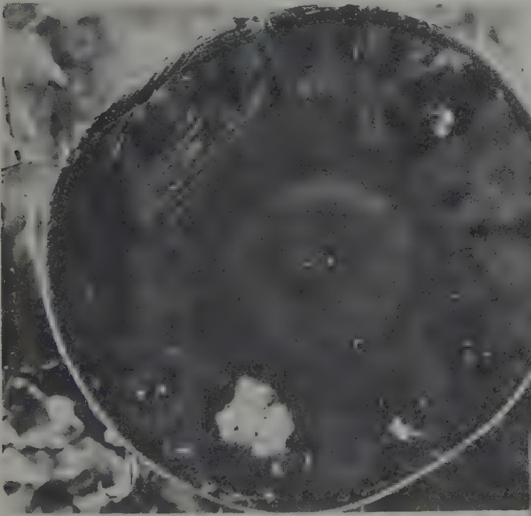

FIGURE 1. (left) The end of a paper birch bolt cut 6 weeks. The white spots (*Cephalosporium* sp.) are beginning to appear on the black patches (*C. coerulescens*). Perithecia of *C. pluriannulata* are scattered between the colonies of *C. coerulescens*.

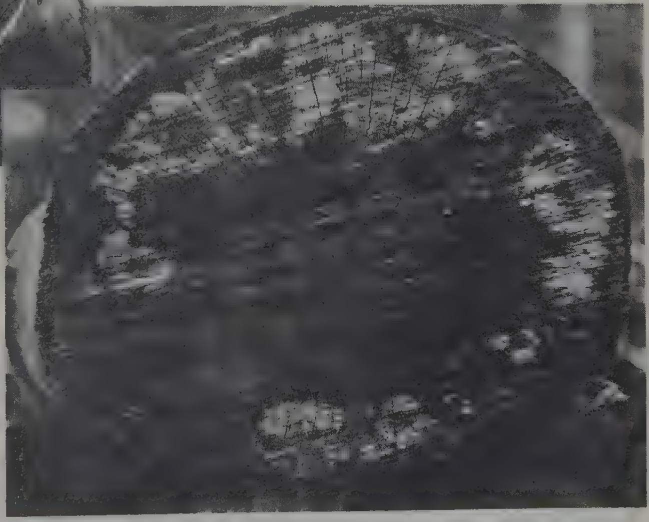

FIGURE 2. (right) The end of a beech bolt cut 6 weeks. Most of the aerial portions of *C. coerulescens* have been appressed by *Cephalosporium* sp.

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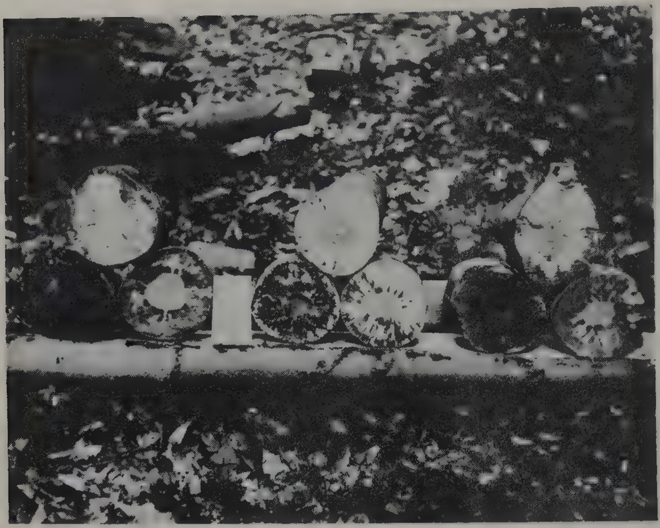

FIGURE 3. Six-inch sections from three beech bolts left on the ground for 5 weeks. The stain had penetrated 18 inches into the bolt. The white spots indicate the presence of the imperfect fungi.

The principal dark fungus was *Ceratocystis coerulescens* (Münch) Bakshi, which also is an important stainer of lumber in the South (1) and the incitant of the sapstreak disease of sugar maple in the Southeast (2). The fungus was isolated from stained areas 18 inches from the ends of some beech bolts that had been on the ground for 5 weeks (Fig. 3). Beech and red maple bolts were invaded more rapidly than the others. The bolts on the bottom of the stacks were infected first, especially those in contact with the ground. The cut surfaces that were exposed to the sun for long periods each day usually were free from obvious fungi. The top bolts stacked in a north-south direction in unshaded areas were seldom infected.

The principal white fungus was *Cephalosporium* sp. The hyphae twined tightly around all the aerial portions of *C. coerulescens*, collapsing them. *Gliocladium roseum* and *Trichothecium roseum* were also found growing over the aerial portions of *C. coerulescens*. Other imperfect fungi were occasionally found.

Ceratocystis piuriannulata and *Graphium rigidum* also grew over the cut surfaces and caused some staining within the bolts. These fungi did not form compact mycelial patches as did *C. coerulescens* (Fig. 1). The expanding white spots were not so obvious on these fungi.

Insects were abundant on the cut surfaces, especially under and around the loosened bark.

DISCUSSION

Ceratocystis coerulescens is an important staining fungus in the Northeast because of the rapidity with which it infects and invades freshly cut logs. It was usually the first fungus seen on the cut ends of hardwood pulpwood bolts.

The regular appearance of certain imperfect fungi on established colonies of *C. coerulescens* indicated that this was the usual succession of fungi on hardwood bolts in the area. The effect of the imperfect fungi on *C. coerulescens* is not known. The many insects on the freshly cut logs probably disseminate most of the spores from one log to another. Once the imperfects get established, their spores are disseminated along with the others. The early establishment of the imperfects on *C. coerulescens* may affect its growth and sporulation. An investigation of this association is being made.

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A NEW PHYTOPHTHORA FRUIT ROT OF ACHRAS SAPOTA FROM INDIA[1]

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Summary

A new Phytophthora fruit rot of chikoo [sapodilla] (Achras sapota), incited by Phytophthora palmivora Butler, is described from India. The pathogen persists through soil, mummies and even seed.

INTRODUCTION

Chikoo [sapodilla] (Achras sapota) has been singularly free from any parasitic diseases so far in India. During August 1955, however, a serious fruit rot of this valuable fruit crop was reported for the first time, from the main chikoo-growing regions of Gholwad and Dahanu of Thana District of the old Bombay state. The disease was favoured by humid weather brought about by heavy rainfall of the tract. A detailed investigation was therefore undertaken on the etiology of the disease, a brief report of which is presented in this note.

SYMPTOMS

The infection was largely confined to the lowermost fruits, resulting in rotting and severe fruit drop (Fig. 1). The fruits higher on the trees were free from infection, indicating that the infection may have originated from the soil below. No other part of the plant showed any infection. Isolations made from infected fruits obtained direct from the tree as well as from the ground invariably yielded a pure culture of a species of Phytophthora; strangely enough even the seeds obtained from heavily infected fruits gave a pure culture of the fungus when planted on oatmeal agar plates.

MORPHOLOGY OF PATHOGEN

The fungus produced abundant papillate sporangia and spherical chlamydospores on host and in culture media, especially of the non-synthetic types. The sporangia measure 31.6-76.6 (53.5)μ in length and 22.2-36.4 (29.3)μ in breadth; chlamydospores 14.2-51.2 (34.4)μ in diameter (Fig. 2).

PATHOGENICITY AND HOST RANGE

The pathogenicity of the isolated organism was successfully established on healthy ripened as well as unripened fruits with and without injury. The first symptoms of the disease appeared 72 hours after inoculation, progressing rapidly under moist chamber conditions and enveloping the entire fruit within 5 days. Half-ripened fruits appeared more susceptible than green fruits.

Besides chikoo, the fungus readily infected fruits of guava (Psidium guajava), banana (Musa sapientum), papaya (Carica papaya), fig (Ficus carica), apple (Pyrus malus), mosambi (Citrus aurantium var. sinensis), chilli (Capsicum annuum), and potato (Solanum tuberosum). It also produced damping-off of seedlings in castor (Ricinus communis), cotton (Gossypium sp.), brinjal [eggplant] (Solanum melongena) and Sesamum indicum under artificial inoculation experiments.

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[1] Part of the work submitted by the senior author to the University of Poona, in fulfillment of the requirements for the degree of Master of Science. Research carried out at Plant Pathology Laboratory, College of Agriculture, Poona, India.

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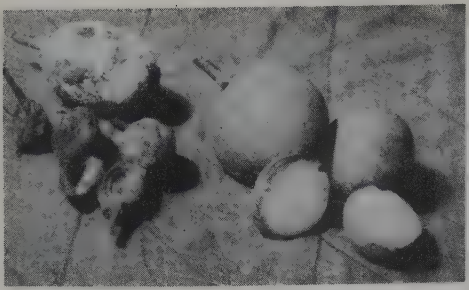

FIGURE 1. Diseased (left) and healthy (right) fruits of chikoo.

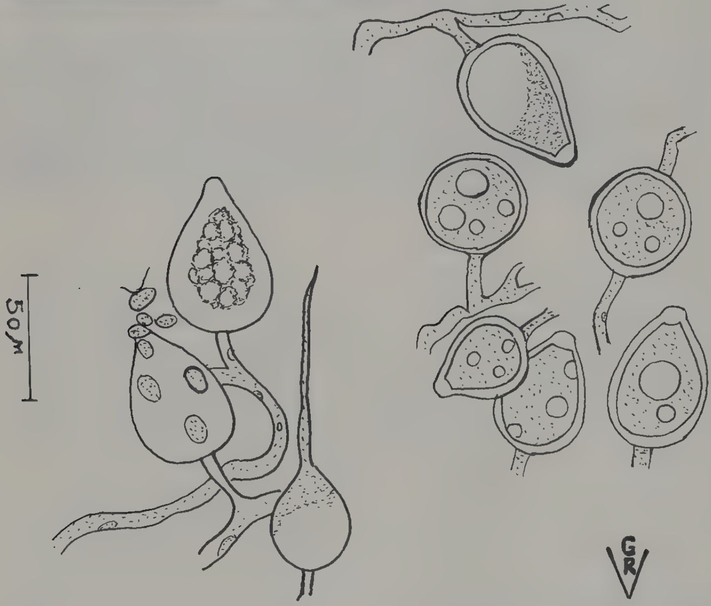

FIGURE 2. Camera lucida drawings of Phytophthora palmivora Butler.

CULTURAL CHARACTERS

The fungus made good growth and produced flat sub-aerial colonies on oatmeal, cornmeal and gram-meal agars and steamed rice, with abundant production of typically papillate sporangia and spherical chlamydospores. It made scanty growth on synthetic media. None of the isolates of Phytophthora obtained from different regions produced oospores in either host tissue or in culture media, or even by pairing the regional isolates in different combinations.

On oatmeal agar the fungus grew best at 26° to 28° C, with minimum at 10° and maximum at 32.5°.

TAXONOMY

On the basis of sporangial measurements and characters, temperature relations and host-range, the fungus agrees with Phytophthora palmivora Butler as described by Tucker (1). A similar disease with identical etiology was reported by Uppal and Kamat (2) from Bombay, producing gummosis and brown rot of mosambi (Citrus aurantium), and incited by the same species of Phytophthora.

The disease is a new record and is perpetuated through soil. Experimental evidence has also been obtained of its hibernation through seed and in the form of "mummies." This is the first record of the occurrence of Phytophthora palmivora Butler on Achras sapota.

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2. UPPAL, B. N., and M. N. KAMAT. 1936. Gummosis of citrus in Bombay. Indian J. Agr. Sci. 6: 803-822.

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DETERMINATION OF NEMATODE MORTALITY[1]

Lawrence M. Fenner[2]

In the enforcement of Foreign Plant Quarantines, nematode infected plant material may be subjected to hot water treatments to eliminate nematodes of plant quarantine importance. To determine the effectiveness of such treatments an accurate, efficient and rapid means of determining the mortality of nematodes and their eggs after treatment is desired. A search for such a method was initiated at the Hoboken Inspection Station, working with adult and immature specimens of several species of parasitic nematodes. Included in the study were *Anguina tritici*, *Aphelenchoides* sp., *Ditylenchus* sp., *Meloidogyne* spp., *Pratylenchus* sp., and *Heterodera trifolii*. This paper reports the results of a series of tests using the dye Phloxine B (C. I. No. 778), to differentiate the living from the non-living nematodes.

MATERIALS AND METHODS

The exact concentration of the phloxine is not important. From a 5% stock solution (aqueous) only a minute quantity is needed to add to the water suspension of nematodes in a Syracuse watch-glass or Petri dish. With the use of a dissecting or compound microscope, confirmation of mortality is indicated by the stained nematodes. The infected host tissues should be dissected to expose the nematodes before the application of the dye. In thin-walled cells of some host material, the nematodes may be stained *in situ*, as is true also of the eggs in the gelatinous matrix of certain genera such as *Meloidogyne*.

OBSERVATIONS

In working with a few genera of nematodes, we found that a weak aqueous solution of phloxine dye does not kill or stain living nematodes and their eggs. In one observation, some specimens of *Ditylenchus dipsaci* remained alive and unstained for as long as 55 days. When killed with higher temperatures (up to 150° F), adults, larvae, and eggs will absorb the dye immediately, staining from light to dark pink. When killed with temperatures used in quarantine treatments (110° F for 4 hours, 118°-120°-122° for 30 minutes, or 127° for 2 minutes), the nematodes will, with certain exceptions such as *Meloidogyne* spp., stain almost immediately after application of the dye. Stain reaction appears to be associated with the withdrawal of the stylet, the relaxing of the orifices, or rupture of the body wall. The majority of treatments are given at 118° to 120° for 30 minutes. Under these conditions the favorable time to apply the dye is 1 to 3 hours after the treatment. The fusiform and filiform dead nematodes should stain immediately, while the pyriform or oval shaped forms assumed by females, for example *Meloidogyne* spp., may not accept the stain for several hours. Inactive moribund larvae will also be slow to accept the stain.

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POSSIBLE USE OF URETHANE FOAM FOR FILLING CAVITIES IN TREES

Curtis May[1]

Concrete has long been the material of choice for filling cavities in trees, but it is not entirely satisfactory for this use. It is rigid, does not accommodate to movements of the tree, and does not make a water-tight seal with wood. Paints are used to prevent entrance of water between wood of the tree and the filling, but paint seals dry and crack eventually. Then water can enter. Freezing of water behind the filling can break the concrete and permit establishment of fungi that cause decay. Also, concrete is heavy. Its advantages are low cost, availability, ease of installation, resistance to vandalism, and durability.

Urethane foam would seem to have possibilities as a material for filling cavities in trees. Some formulations will bond firmly to wood, are sufficiently flexible to accommodate to movements of tree parts, and present no weight problem. In a preliminary *in vitro* test with ten species of woodrotting fungi, a urethane foam showed no visual evidence of deterioration after 6 months' exposure to the fungi. A urethane foam formulation showed no evidence of surface deterioration 10 months after installation in a cavity in red oak. Callus formed at the rim of the wound. Much further research is required in evaluation of urethane foam as a material for filling cavities in trees.

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[1] Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

CORRECTION

REPORTER, April issue (Volume 46, Number 4), page 214. In Table 1, change the fourth line from the bottom to read: 2 (I. J.) : 1 *milk*, instead of 2 (I. J.) : 1 *water*.

THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 46 Number 6

June 15, 1962

1862 1962

Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

COMMONWEA
6 JUL 1962

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Paul R. Miller

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THE PLANT DISEASE REPORTER

Crops Research Division
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Plant Industry Station, Beltsville, Maryland
Number 6

June 15, 1962

CONTENTS

1. Streptomycin resistance of the bacterial spot pathogen and control with streptomycin
R. E. STALL and P. L. THAYER 389

2. Forecasting downy mildew of lima bean in Cape May County, New Jersey
R. A. HYRE, et al. 393

3. Fusarium wilt of cotton moves west
L. M. BLANK 396

4. Six years of testing streptomycin - oxytetracycline for control of fire blight on Bartlett pear
HARRY L. KEIL and ROY A. WILSON 397

5. Results of three treatments for the control of Dutch elm disease
LAWRENCE R. SCHREIBER and JAMES M. HARRISON 401

6. Influence of gibberellin on bud differentiation of sour cherry and on damage by sour cherry yellows
WLADYSLAW BASAK, et al. 404

7. Studies on the host range of watermelon mosaic virus in Hawaii
H. HAROLD TOBA 409

8. New physiologic races of bean rust, Uromyces phaseoli typica, from Mexico
ALFONSO CRISPIN and SEGUNDO DONGO 411

9. Cucumber, an additional herbaceous host of the infectious variegation strain of citrus psorosis virus
PAUL R. DESJARDINS and JAMES M. WALLACE 414

10. Effect of removal of eggs from cysts on hatching of larvae of Heterodera schachtii
ARNOLD E. STEELE 417

11. Sclerotium rolfsii in Israel
ZEHARA AVIZOHAR-HERSHENZON and J. PALTI 418

12. Effects of sting and stubby-root nematodes on St. Augustine grass
H. L. RHOADES 424

13. Effects of big vein on the irrigated head lettuce crop
R. B. MARLATT and R. T. McKITTRICK 428

14. Evaluation of chemicals for systemic action in citrus seedlings infected with Radopholus similis
A. W. FELDMAN and R. W. HANKS 430

15. Fungicidal control of apple blotch, Marssonina mali (P. Henn.) Ito, in South Korea
KWANG YUN LEE and CHONG CHON KIM 435

16. Reactions of winter oats to soil-borne mosaic
F. A. COFFMAN, et al. 438

17. Evaluation of treatments for control of soil-borne pests in celery seedbeds
J. F. DARBY, et al. 441

18. Does the corn leaf blight fungus survive Wisconsin winters?
PAUL E. HOPPE 444

19. Sting nematode on grape cuttings in Florida
N. C. SCHENCK, et al. 446

20. Brooming and galling of shagbark hickory in Arkansas
CHARLES L. WILSON 448

21. The occurrence and distribution of the citrus-root nematode, Tylenchulus semipenetrans Cobb, in Florida
CHANCELLOR I. HANNON 451

22. An agitator-incubator for aseptic culturing of virus-infected cells and tissue in liquid media
H. H. THORNBERRY 456

23. A microvolumeter for measuring the volume of plant tissue cultures
H. H. THORNBERRY and INEZ N. KAMP 459

24. Tobacco frogeye control in Venezuela by isolation of plant beds from native vegetation
W. D. VALLEAU 461

25. Reduction in yield of cotton caused by diseases in 1961
COTTON DISEASE COUNCIL 462

26. The occurrence of Dutch elm disease in Oklahoma
JOHN E. THOMAS and CLYDE A. BOWER 463

STREPTOMYCIN RESISTANCE OF THE BACTERIAL SPOT PATHOGEN AND CONTROL WITH STREPTOMYCIN

R. E. Stall and P. L. Thayer[1]

Summary

The proportion of streptomycin-resistant isolates of the bacterial spot pathogen (Xanthomonas vesicatoria (Doidge) Dows.) increased after field sprays of streptomycin in one test but not in another. Control of the disease was nullified by the increase of the resistant bacteria. A streptomycin-susceptible isolate was controlled in greenhouse tests with streptomycin, but a resistant isolate was not.

Bacterial spot (Xanthomonas vesicatoria (Doidge) Dows.) causes severe damage to tomato and pepper. Hundreds of acres of these crops in Florida have been abandoned because of this disease, although copper and streptomycin were used bi-weekly or even more frequently.

The results of numerous tests on control of bacterial spot with streptomycin were summarized by Thayer and Stall (3). This summary pointed out the erratic nature of control with this material. These authors found strains of the causal organism differing in susceptibility to streptomycin and found this variation to be important in field control. Questions were raised, however, as to whether streptomycin-resistant populations would increase in relation to streptomycin-susceptible populations on plants sprayed with streptomycin. An excellent opportunity developed during the fall of 1961 to evaluate effects of streptomycin sprays on the causal bacterium in field plots, and the results are reported here. This paper also presents results of greenhouse tests on control of streptomycin-resistant and -susceptible isolates with streptomycin sprays.

FIELD CONTROL AND STREPTOMYCIN RESISTANCE

Two field tests, one with tomato at Ft. Pierce and the other with pepper at Belle Glade, were completed in the fall of 1961. Ratings of disease incidence on streptomycin-sprayed plants were made and streptomycin resistance of isolates from these sprayed plants was determined in laboratory tests.

Methods: Sprays of streptomycin (AS-17, 17% streptomycin sulfate) were applied to plants in field plots arranged in randomized block experimental designs. Sufficient volumes of spray were delivered under adequate pressure to wet the foliage thoroughly.

The degree of resistance to streptomycin was evaluated on Nutrient Agar or King's Medium B[2] to which a concentration of 0, 100, 200 or 400 ppm streptomycin was added after cooling to about 50°C. A bacterial suspension obtained from a lesion crushed in approximately 1 ml of sterile tap water was streaked onto media containing each concentration of streptomycin. Growth of the bacterial spot pathogen on the media was rated plus or minus.

Many types of bacteria were isolated by this procedure. Only a particular type, as characterized by color and growth, was rated. This type did not cause fluorescence on King's Medium B and did not acidify a glucose-mineral base-broth in 24 hours. It grew extremely rapidly, but did not clear a yeast extract - $CaCO_3$ - glucose medium[3]. Colonies of this type have been pathogenic on tomato and pepper in many tests.

Results: Field Control on Tomato. Bacterial spot developed abundantly in this test. The primary source of the causal organism was from diseased volunteer tomato plants that had survived the summer after a spring tomato crop. The organism was disseminated to seedlings from volunteer plants which were placed 5 feet apart on the rows before seedling emergence.

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[1] Assistant Plant Pathologists, Everglades Experiment Station at Indian River Field Laboratory, Ft. Pierce and Belle Glade, Florida, respectively. Florida Agricultural Experiment Station Journal Series No. 1431.

[2] King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanins and fluorescin. J. Lab. and Clin. Med. 44: 301-307.

[3] The authors wish to thank Dr. M. P. Starr, Department of Bacteriology, University of California, Davis, California for valuable help in identification of the bacterial spot pathogen. Dr. Starr suggested screening bacterial isolates in this manner.

Sprays were applied on a 4 to 5 day schedule, beginning 4 days after emergence of seedlings, except when additional sprays were applied after each rain. Ten sprays were applied during the 34 days of the tests.

Three disease ratings were made (Table 1). In the first two ratings, good control was obtained with streptomycin and there was no significant difference in control with the different concentrations. At the time of the third rating, however, there was no significant control of the disease by any concentration of the antibiotic material.

Streptomycin Resistance of Isolates from Tomato. After the second rating, when streptomycin appeared to control the disease, 35 lesions were collected from unsprayed plants and the bacteria from them were streaked onto streptomycin agar. Only 8% of the isolates were resistant to 100 ppm streptomycin. This 8% was also resistant to 400 ppm.

After the third rating, when streptomycin did not control the disease, bacteria were isolated from 20 lesions collected from each treatment and tested for resistance to streptomycin. A striking difference in the amount of resistant isolates on streptomycin-sprayed plants versus unsprayed plants occurred (Table 1). There was little difference in the amount of resistant isolates on plants sprayed with the different concentrations of streptomycin. This same relationship held in control of the disease earlier in the test.

Field Control on Pepper. Bacterial spot developed abundantly in this trial. The causal organism became established in the plots naturally even though the plots were at least 5 miles from other pepper or tomato plantings. Spray materials were applied twice weekly. Twenty-nine spray applications were made during 97 days of the test.

Several ratings were made, but only three representative ratings are listed in Table 2. In every rating, good control of bacterial spot was noted. The difference in control with the different concentrations of streptomycin was not statistically significant.

Streptomycin Resistance of Isolates from Pepper. After the December 8 rating, bacteria were isolated from 12 lesions on plants from each treatment and tested for resistance to streptomycin. All bacteria isolated were susceptible to 100 ppm streptomycin (Table 2). The typical bacterial spot pathogen was not isolated from lesions on plants sprayed with 400 ppm streptomycin and from only one lesion on plants sprayed with 200 ppm. The bacterium was isolated from all lesions from unsprayed plants and from plants sprayed with other materials included in the test. Bacteria other than X. vesicatoria could possibly have caused the lesions on the streptomycin-sprayed plants. However, 20 pepper plants were inoculated with the types of bacteria isolated from the lesions, and there was no disease development.

GREENHOUSE CONTROL AND STREPTOMYCIN RESISTANCE

In preliminary work, many isolates that differed in streptomycin resistance were found to be pathogenic, which indicated resistance to streptomycin was independent of pathogenicity. Two isolates from field plots (Fig. 1), one susceptible to 100 ppm and one resistant to 400 ppm streptomycin in agar, were tested for differences in control by streptomycin sprays on greenhouse plants.

Methods: Inoculum of each isolate was derived from a single clone which was increased on lima bean agar slants. One loop of bacteria from a slant was placed in 10 ml of sterile tap

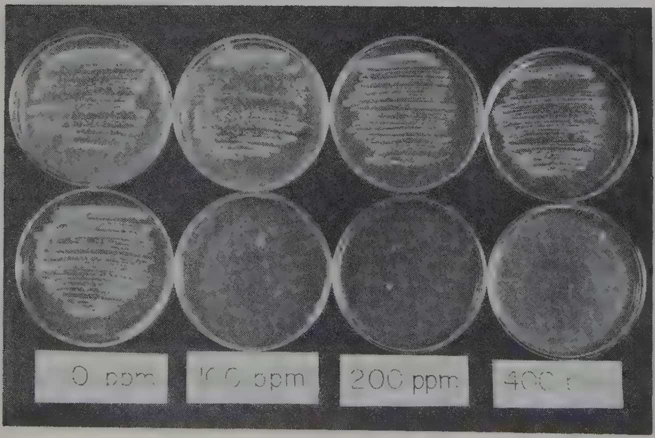

FIGURE 1. Growth of the bacterial spot pathogen on a medium with indicated ppm of streptomycin sulfate added. Top, streptomycin resistant; bottom, streptomycin susceptible.

Table 1. Control of bacterial spot and development of resistant isolates on tomato at Ft. Pierce.

Streptomycin[a] treatments (ppm)	Bacterial spot index[b] rating dates			% isolates on 11-10-61 which grew on indicated ppm streptomycin in medium			
	10-16	10-25	11-8	0	100	200	400
0	6.5	5.3	6.5	100[c]	25	15	0
100	2.8	2.3	6.0	100	85	70	15
200	3.3	2.8	5.8	100	85	80	25
400	2.3	2.0	5.8	100	100	90	25
SSR[d] .05	1.6	0.9	NS				
SSR .01	2.3	1.3	NS				

[a] Source of streptomycin was AS-17, 17% streptomycin sulfate.

[b] Index based on a 0-10 scale where 0 equals no disease and 10 equals at least one lesion on all leaflets. Average of four replicates.

[c] % based on 20 isolates.

[d] SSR is Significant Studentized Range based on Duncan's Multiple Range test.

Table 2. Control of bacterial spot and development of resistant isolates on pepper at Belle Glade.

Streptomycin[a] treatments (ppm)	Bacterial spot index[b] rating dates			% isolates on 12-1-61 which grew on indicated ppm streptomycin in medium			
	10-30	11-8	11-29	0	100	200	400
0	3.7	5.0	5.5	100[c]	0	0	0
200	2.2	3.2	3.2	8	0	0	0
400	1.5	2.7	2.2	0[e]	0	0	0
SSR[d] .05	1.2	0.9	1.1				
SSR .01	1.6	1.2	1.4				

[a] Source of streptomycin was AS-17, 17% streptomycin sulfate.

[b] Index based on a 1-12 scale where 1 equals no disease and 12 equals dead plants. Average of four replicates.

[c] % based on 12 isolates.

[d] SSR is Significant Studentized Range based on Duncan's Multiple Range test.

[e] No typical bacterial spot pathogen growth even though no streptomycin in agar.

Table 3. Lesions per leaf on plants sprayed with streptomycin and inoculated with streptomycin-resistant and -susceptible isolates.

Type of isolate[b]	ppm streptomycin sprayed on plants			
	0	100	200	400
Resistant	176[a]	167	153	210
Susceptible	186	158	73**	52**

[a] Lesions on third leaf from base of plant. Average of four replicates.

[b] Resistant means resistant to 400 ppm streptomycin in agar plates and susceptible means susceptible to 100 ppm streptomycin in agar plates.

** Significantly different at 1% level.

water. One ml of this suspension was then placed in a Petri dish of lima bean agar. A 3-day-old Petri dish culture suspended in 100 ml of tap water was sprayed on plants until run-off. Care was taken to cover tops and undersides of each leaf.

Eight tomato plants, with the fifth leaf in the bud stage, were sprayed with 0, 100, 200 or 400 ppm streptomycin 3 days and 1 day before inoculation. Half of the plants that were sprayed with each concentration were inoculated with the susceptible or the resistant isolate. Plants were held in a moist chamber for 7 days, then removed to a greenhouse bench and rated for disease 17 days after inoculation. Lesions were counted on the third leaf from the base of the plant.

Results: Streptomycin sprays resulted in less disease on plants inoculated with the streptomycin-susceptible isolate than on plants inoculated with the streptomycin-resistant isolate (Table 3). Sprays of 200 and 400 ppm streptomycin controlled the susceptible isolate, but not the resistant isolate. Although the susceptible isolate was susceptible to 100 ppm streptomycin incorporated in the media, two sprays of 100 ppm did very little in controlling the disease on the plants.

Difficulty exists in interpreting the concentration of streptomycin on plants after spraying, but it is important that the susceptible isolate was controlled by streptomycin on the plant and the resistant isolate was not controlled. This would indicate that predictions of whether a farmer could control the disease in his field might possibly be given on the basis of results of plate tests.

DISCUSSION

The effect of streptomycin sprays on the bacterial spot pathogen is interesting. In one test a predominantly streptomycin-resistant population developed from a population of apparently low percentage of resistant individuals. In the other test, no resistant bacteria were found. This is evidence that the buildup of resistant bacteria was dependent upon a mixed population. Thus, the mechanism of this buildup was apparently one of "selection" rather than one of " adaptive" change of the bacteria in the presence of streptomycin.

A predominantly resistant population developed on tomato after only 10 sprays. The length of time for the increase of resistant bacteria to the point of no control is undoubtedly influenced by many factors, but the rapidity of the development in this test could mean that streptomycin is extremely effective against susceptible isolates under field conditions.

The fact that the bacterial spot pathogen could not be isolated from pepper plants sprayed with 400 ppm streptomycin and from only one lesion from plants sprayed with 200 ppm streptomycin suggests near-eradication of the organism from the lesions. There was no evidence of eradication of the organism in the tomato test or in greenhouse tests by Crossan and Krupka (1). However, among other factors, the susceptibility of the bacterium to streptomycin would undoubtedly be a factor in eradication of the organism. The bacteria that caused the lesions on pepper could possibly have been susceptible enough that eradication occurred at the rates of streptomycin used.

All isolates tested were obtained from plants in the field. Thus, resistant strains can survive and remain virulent from season to season. However, it remains to be determined whether resistant isolates can compete as well as susceptible isolates over a long period of time. If they could not, streptomycin may possibly be more effective in the future in areas where its use has been discontinued.

This work is further evidence that streptomycin resistance of the causal organism is a factor in explaining erratic results with streptomycin sprays. This variation is probably the most important reason for the widespread failures to control the disease in Florida. Resistant bacteria could have caused failures to control the disease early in evaluations of streptomycin, such as reported by Darby (2). Through greater use of the antibiotic, a predominantly resistant population may have been selected. Investigations of the amount of resistance in areas where the antibiotic has been used extensively versus areas where it has been used sparingly should provide evidence for this.

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FORECASTING DOWNY MILDEW OF LIMA BEAN IN CAPE MAY COUNTY, NEW JERSEY[1]

R. A. Hyre, John MacLeod, and Spencer H. Davis, Jr.[2]

Abstract

Forecasts of downy mildew of lima bean were made for Cape May County, New Jersey for 5 years. Three methods, involving the use of criteria-favorable days, severity values, and Beaumont periods, were compared. The forecasts were quite accurate, especially for the incidence of disease. Those made from the use of the first two methods were in general agreement and more accurate than those made from Beaumont periods.

Downy mildew of lima bean, caused by Phytophthora phaseoli, was present in northern New Jersey as early as 1897 (4). In subsequent years it profoundly affected the lima bean processing industry. A 1948 evaluation of its importance, at a meeting called by Seabrook Farms, showed that it had also become a major agricultural problem in southern New Jersey. For example, because of this disease Seabrook Farms abandoned over 1200 acres of beans in 1946, and their estimated loss in 1948 was over $454,000.00 (1).

As a result of the 1948 conference at Seabrook Farms, the senior author attempted to devise a method for forecasting downy mildew. Disease records of Seabrook Farms were correlated with rainfall and temperature data obtained from the United States Weather Bureau. Later, in Cape May County, relative humidity and temperature data also were related to disease incidence.

The study reported here covered a period of 5 years, 1957 through 1961. In 1957, George Mingin, a former agricultural agent, made disease observations and collected weather data. The authors are indebted to him for that service. During the last four years Agricultural Agent MacLeod made these observations.

MATERIALS AND METHODS

Three methods were studied for use in making the forecasts. The first method was based upon rainfall and temperature data (5). Briefly, the incidence of disease was predicted after 8 consecutive days when the 5-day mean temperature was less than 79° F and the concurrent 10-day cumulated rainfall was 1.20 inches or more, provided the weather forecast at that time was for continued mildew-favorable weather.

The second method utilized relative humidity and temperature data obtained from sheltered 7-day recording hygrothermographs placed in or near the fields. Mildew severity values were derived from the instrument charts in a manner similar to blight severity values as derived by Wallin[3]. The conversion table is reproduced here (Table 1). A potential infection period was disregarded if the maximum temperature the following day exceeded 85° F (6).

The total weekly mildew severity value that would warrant repeat applications of a fungicide, once mildew was present, was unknown when this study was initiated. The corresponding blight severity value employed by Wallin is 3. Also, Wallin uses a cumulated severity value of 20 from the time of emergence of the potatoes as a zero point before which blight is not expected.

The third method indicated a positive forecast of mildew after 48 or more consecutive hours of relative humidity of 75% or more. These are known as Beaumont periods (2). The temperature range can be assumed to be the same as in the second method. In this third method a self-calculating blight forecast recorder (3) was used.

The instrument charts and other meteorological data, along with disease records, were forwarded at weekly intervals by the agricultural agent to Newark, Delaware, where the senior author then made the mildew forecasts. In addition, the agricultural agent analyzed the weather data and, at critical times especially, formulated his own forecasts and proceeded accordingly before receiving the mildew forecasts from Delaware.

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[1] Report of a cooperative investigation of the New Jersey Agricultural Experiment Station and the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, The Pennsylvania State University, University Park, Pennsylvania.

[2] Plant Pathologist, Crops Research Division, United States Department of Agriculture, The Pennsylvania State University, University Park, Pennsylvania; Agricultural Agent, Cape May County, New Jersey; Extension Specialist in Plant Pathology, Rutgers University, New Brunswick, New Jersey.

[3] Wallin, Jack R. 1957. Plant Disease Situation Report 28, U. S. Dept. of Agr. (Mimeo.)

Weather data were analyzed from the Cape May, Cape May Court House, and Seaville areas of the county. The distance between stations was about 10 miles. A self-calculating recorder was in operation only at Cape May.

Table 1. Conversion table for determining disease severity values.

Temperature range (° F)[a]	Mildew severity values 1	2	3	4
	Hours			
45-53	16-18	19-21	22-24	25-
54-59	13-15	16-18	19-21	22-
60-80	10-12	13-15	16-18	19-

[a] The mean temperature when the relative humidity is $\geqq$ 90%.

RESULTS

Figure 1 shows the analyses of the weather data, the disease forecasts, and the occurrence of the disease.

The disease forecasts were surprisingly accurate. At Cape May in 2 years, on September 11, 1957 and July 24, 1959, mildew was first observed just 8 days after the first positive forecast. On July 3, 1961 mildew was seen 12 days after the first positive forecast. In 1958 there was a change in agricultural agents and the program was late in getting started. In that year severity values were not obtained due to a malfunctioning hygrothermograph, and the first disease forecast was made after mildew was present. The disease was quite severe when first reported on July 21 and was estimated to have been present about 2 weeks. This would have placed the first occurrence about 10 to 12 days after the first recorded period of 8 criteria-favorable days. In 1960 mildew appeared early (June 14) at Cape May and before any disease forecasts were made. However, a 10-day criteria-favorable period ended 13 days before mildew was found.

At Seaville disease forecasts were made for 2 years, but early weather records were not at hand in 1960. That year mildew was found on July 11, before the first positive forecast for that area. It was found 19 days after the end of a criteria-favorable period at Cape May Court House where earlier records were available. In 1961 mildew was found on July 10, at Seaville, 12 days after the first unqualified positive disease forecast. Through the years not only the incidence of mildew but increases in its severity were in good agreement with the forecasts.

The first two forecast methods, criteria-favorable days and mildew severity values, were used for making the disease predictions. The two methods were in general agreement except late in the season when severity values were recorded without criteria-favorable days and without an increase in the incidence of mildew. Also, the magnitude of severity values has still to be satisfactorily resolved both for incidence and increase in severity of the disease. The number per week required for an increase in mildew would appear to be somewhat higher than the three suggested for the late blight disease.

The 75% relative humidity method (Beaumont periods) was unsatisfactory for predicting mildew. There was such a period before the incidence of mildew in 1959 but not in 1958 or 1961. In 1960 mildew was found before the self-calculating recorder was put in operation. In addition, the increase in severity of mildew was not well correlated with additional Beaumont periods.

DISCUSSION

As indicated earlier, the mildew forecasts were more accurate than originally anticipated, especially for incidence of the disease. They were more accurate than the forecasts made by the senior author for the late blight disease of potato. This was probably due in part to an ever present source of inoculum, since the fungus produced abundant oospores.

The system of predicting downy mildew disease of lima bean utilizing the criteria-favorable day method has been especially successful in forecasting initial outbreak of the disease, and also has led the way to development of a similar technique for predicting late blight of potato. However, more precise information is still needed in order to forecast fluctuations in severity once a disease is present. Since such information can best be obtained by working with the disease under controlled conditions, this phase of the work was recently undertaken.

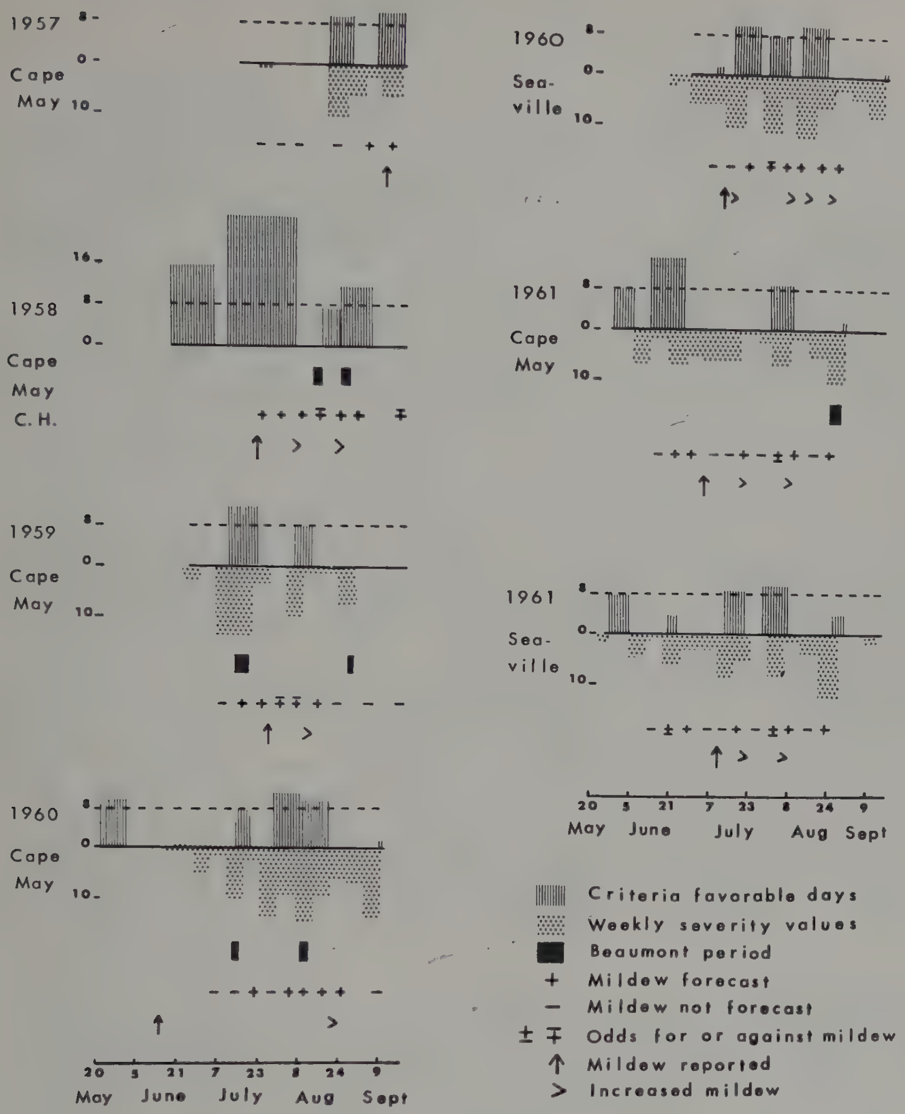

FIGURE 1. An analysis of weather data by three methods, forecasts of downy mildew, and the occurrence of the disease in Cape May County, New Jersey for the years 1957-1961.

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FUSARIUM WILT OF COTTON MOVES WEST[1]

L. M. Blank[2]

Fusarium wilt of cotton, caused by Fusarium oxysporum Schlecht. f. vasinfectum (Atk.) Snyd. & Hans., has been recognized for many years as a major disease of cotton on the acid soils in the rainbelt production area of the United States. This disease was reported recently[3] on sandy soils with a pH near 8 in five counties of west and northwest Texas, near the New Mexico state line. The purpose of this note is to report its occurrence on Upland cotton (Gossypium hirsutum) near Mexicali, Baja California, Mexico, immediately to the south of the Imperial Valley cotton production area of California.

Symptoms of a wilt disease of Upland cotton in the Mexicali area were first observed in 1955, and by 1961 had been noted in a number of fields[4]. The symptoms consisted of internal discoloration of stem tissues, burning and necrosis of leaf margins, shedding of leaves, and stunting or necrosis of plants. Root-knot nematode damage was commonly associated with these symptoms. The soil type is described as a silty loam with a pH range of 8.1 to 8.3 and a fairly high salt content.

Shipments of diseased cotton plants, variety Deltapine Smooth Leaf, were received from the Mexicali area in June, July, and August 1961. Tissue platings on several media consistently yielded a species of Fusarium. Pathogenicity of isolates from all three lots of plants was demonstrated on seedling plants of several varieties of Upland cotton and the causal organism recovered. Comparative studies with identified isolates of F. oxysporum f. vasinfectum from Mississippi and Texas demonstrated a general similarity of performance of all cultures on a number of agar media and also in regard to pathogenicity on two varieties of Upland cotton. A culture of the Fusarium from Mexicali was submitted to W. C. Snyder, who reported it to be "a typical sporodochial clone of the species"[5] (F. oxysporum f. vasinfectum).

The fact that Fusarium wilt of cotton has become established in a number of fields in the Mexicali area since it was first observed in 1955 suggests that it may be present also in cotton-producing areas between Mexicali and the known locations in west and northwest Texas. The common association of Fusarium wilt and the cotton root-knot nematode has been recognized for many years on the coarse-textured soils in the rainbelt production area. Coarse-textured soils and root-knot nematodes are not uncommon in the irrigated cotton production areas of New Mexico, Arizona, and the Imperial Valley of California. These soils are alkaline, but alkalinity appears to be less of a barrier to the spread of the Fusarium wilt organism than was once believed.

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[2] Pathologist, Crops Research Division, Tempe, Arizona.

[3] Bird, L. S. 1962. The enemy under ground. The Cotton Trade Journal and Agricultural Reporter, International Edition, pp. 64, 88.

[4] This problem was brought to my attention by R. E. Beckett, Agronomist, Granjas del Pacifico, S. de R. L., Mexicali, B. C., Mexico. His cooperation in furnishing plant material and information on field occurrence, soil analyses and other pertinent observations is gratefully acknowledged.

[5] Snyder, W. C. 1961. Personal communication.

SIX YEARS OF TESTING STREPTOMYCIN - OXYTETRACYCLINE FOR CONTROL OF FIRE BLIGHT ON BARTLETT PEAR[1]

Harry L. Keil and Roy A. Wilson[2]

Summary

Antibiotic sprays for control of fire blight (Erwinia amylovora) on Bartlett pear were tested in the field for six consecutive years at Beltsville, Maryland. An antibiotic formulation consisting of 15% streptomycin sulfate plus 1.5% oxytetracycline was used at a dosage rate of 100 ppm. The antibiotic was applied four times (two bloom and two cover sprays) in each of 5 years. Eight sprays were applied in the sixth (last) year. The spray program was supplemented by periodic inspections of the trees, when blighted twigs were pruned out and destroyed. Epiphytotic blight during 2 test years provided exceptionally good test conditions. During these 2 years the average untreated tree had 21 to 23 times as many infected twigs as did the average antibiotic-sprayed tree. No antibiotic residue was found in or on pear fruits harvested 53 days after the last spraying and held for 64 days in cold storage.

INTRODUCTION

Pear growing in the northeastern and midwestern United States except in a few isolated areas has been abandoned because of the ravages of fire blight (Erwinia amylovora (Burr.) Winslow et al.). Preliminary investigations by several workers demonstrated significant reduction in fire blight on apple and pear trees treated with antibiotics (1, 2, 3, 4, 5). Because of these promising results it was thought the use of antibiotics supplemented by pruning out blighted twigs might make it possible to grow quality pears in areas where they once thrived. With this thought in mind the studies reported herein were undertaken.

MATERIALS AND METHODS

To provide fire blight-susceptible trees for these studies, 51 Bartlett pear whips, 1-year old, were planted in May 1954 at the Plant Industry Station at Beltsville, Maryland. The trees were planted 15 feet apart in 5 rows with 20 feet between the rows. During the first and second growing seasons no protection except one application of 100 ppm Agrimycin 100 on April 19, 1955 (full bloom) was given.

Actual testing of the antibiotic got underway in 1956. The antibiotic formulation (Agrimycin 100) consisting of 15% streptomycin sulfate plus 1.5% oxytetracycline (Terramycin) was used throughout at 100 ppm, hereafter referred to simply as antibiotic.

During 1956-60 four spray applications of antibiotic, two bloom and two post bloom, were made from the ground with a hand gun at a pressure of 500 psi. In 1961 a total of eight spray applications (two bloom and six covers) were made.

The trees were kept growing vigorously by a spring application of nitrate of soda or urea. When the trees were young only two or three handfuls of fertilizer per tree were used, but as they grew older as much as 2 pounds of urea per tree was applied.

Periodic inspections of the trees were made throughout each growing season. Infected twigs or branches were removed with pruning shears sterilized between cuts with bichloride of mercury (1:1000). If possible, the cuts were made 8 to 12 inches below the visible infected area. All infected clippings were carried out of the orchard and destroyed.

To control insects, all the trees were uniformly sprayed with parathion. Sprays were applied with a hand gun at a pressure of 500 psi. All insecticide sprays were made separately.

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[1] We thank Chas. Pfizer & Co., Inc., for supplying gratis Agrimycin 100 used. This acknowledgment does not however constitute an endorsement for this product.

[2] Respectively, Plant Pathologist and Agricultural Research Technician, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. J. C. Dunegan and Wilson initiated and conducted the studies through 1958. After Mr. Dunegan's retirement his duties were taken over by Keil.

RESULTS AND DISCUSSION

During 1956-58 all 51 trees in the orchard were uniformly sprayed with antibiotic. Although fire blight was observed in an apple orchard about 75 feet from the pears, no blight appeared in the latter during these 3 years.

In 1959, six trees selected at random were left untreated. The remaining trees were sprayed four times, on April 16 (5% bloom), April 20 (full bloom), May 4 (fruit 0.5 inch in diameter), and May 14 (fruit 0.75 inch in diameter). One check and one antibiotic sprayed tree was artificially inoculated by spraying on May 7 with a water suspension of Erwinia amylovora plus pyrophyllite.

During 1959 data in Table 1 show that 33% of the check trees and 22% of the antibiotic sprayed trees became infected: one infected tree of both check and antibiotic-treated trees might have resulted from artificial inoculation on May 7. Infection in remaining trees, however, apparently resulted from natural causes. Although we did not consider 1959 a particularly bad blight year the untreated trees averaged about six times as many infected twigs as the antibiotic sprayed trees.

Table 1. Fire blight control of Bartlett pear by spraying with 100 ppm of Agrimycin 100.

Year	Treatment	Number of trees	Number of blighted trees	% trees blighted	Av. number of pruning cuts per tree
1959	None	6	2[a]	33	7.5
	Antibiotic	45	10[a]	22	1.2
1960	None	6	6	100	134.5
	Antibiotic	42	29	69	5.9
1961	None	5	5	100	321.0
	Antibiotic	5	3	60	15.0

[a] One tree artificially inoculated by spraying with water suspension of Erwinia amylovora plus pyrophyllite on May 7, 1959.

Three trees died during the winter of 1959-60. Six trees, not previously used as checks, selected at random were allowed to remain untreated during 1960. Forty-two trees were sprayed with antibiotic on April 18 (20% bloom), April 22 (95% bloom), May 4 (fruit 0.25 inch in diameter), and May 17 (fruit 0.75 inch in diameter). Because of severe blight during this year good test conditions were provided.

All checks, but only 69% of the antibiotic trees, blighted during 1960 (Table 1). The average check tree exhibited 135 infected twigs, compared with only 6 per antibiotic tree. In other words, the checks showed, on an average, about 23 times as many blighted twigs as the antibiotic trees. Result of the drastic pruning required on check trees is contrasted with little pruning on antibiotic sprayed trees (Fig. 1). The check trees were reduced to about 1/4 to 1/3 original size by the pruning.

In 1960 blighted twigs were pruned out 14 times (Fig. 2). Apparently much infection took place on the check trees early in the season. In fact, about 73% of the infected twigs on the check trees were pruned out by June 3. A sharp drop in the number of infected twigs took place on these trees between June 3 and 10 and then a gradual reduction until the end of the season is noted. It would have taken until the first week in July to prune a comparable percentage of infected twigs from the antibiotic trees.

Also, the antibiotic sprayed trees averaged less than one blighted twig per tree at any one inspection period (Fig. 2). The curve is very flat, suggesting that controlling fire blight early in the season makes it less of a problem later.

Studies through 1960 indicate that considerable infection occurred following bloom. In fact, our records indicate 41% of the infected twigs in 1960 were associated with succulent terminal growth. This postbloom infection makes it necessary to protect the trees during this period. It was therefore decided to protect the trees to within about 50 days of harvest in 1961.

During 1961 five trees (not previously used as checks) selected at random were left untreated and five trees were treated with antibiotic. Eight applications of antibiotic were made during this season on April 21 (5-10% bloom), April 25 (full bloom), May 2 (calyx), May 16

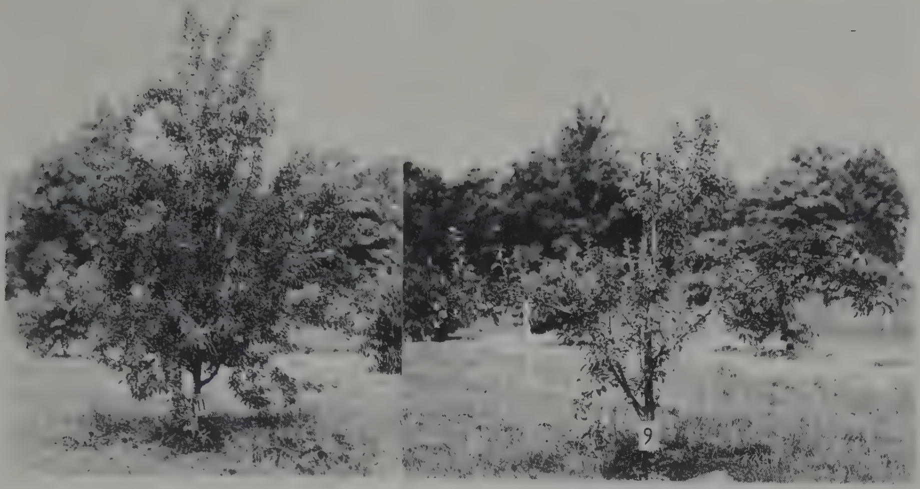

FIGURE 1. Bartlett pear trees showing the effect of pruning out blight infected branches during 1960. Left -- sprayed with antibiotic. Right -- unsprayed.

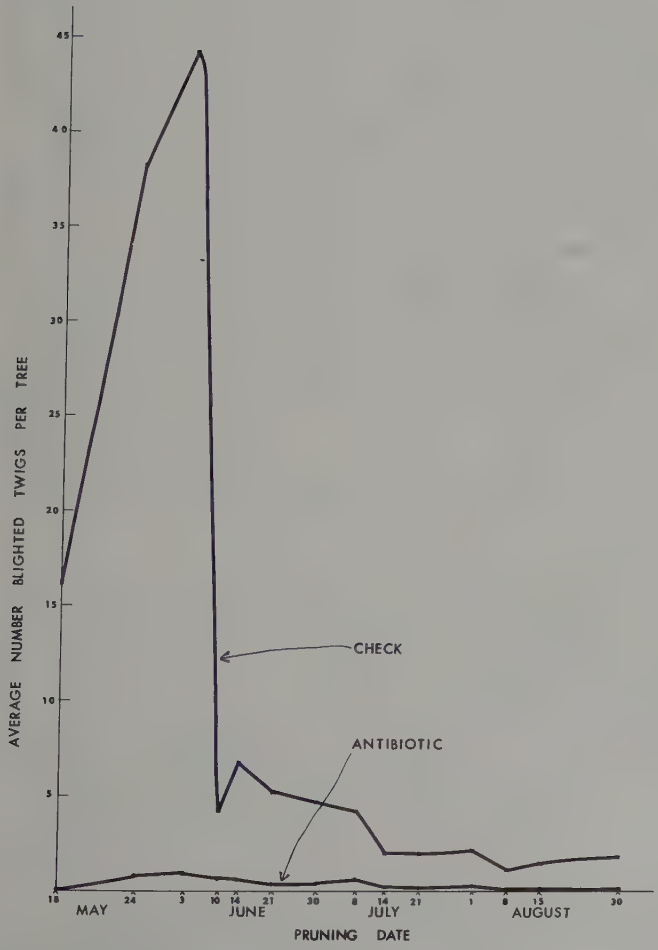

FIGURE 2. Average number of blighted twigs pruned out of untreated and antibiotic sprayed Bartlett pear trees during the 1960 season.

(fruit 0.5 inch in diameter), May 25 (fruit 0.75 inch in diameter), June 5, 22, and 30.

During 1961, data in Table 1 show 321 infected twigs on the average check tree and only 15 on the average antibiotic treated tree. Although more infected twigs occurred in 1961 than in 1960 on both the check and antibiotic treated trees, the ratios are about the same. These results indicate that the antibiotic gave as good control under the more severe blight condition in 1961. It should however be pointed out that four additional sprays were required to give equivalent control under the more severe blight conditions.

An attempt was made in 1961 to stop development of blight infection on the check trees by spraying them with antibiotic on May 16, May 25, and June 5. These sprays however apparently had little effect on development of the disease, suggesting one cannot depend upon this antibiotic formulation to eradicate infection.

In 1961, as in 1960, much blight infection took place during the postbloom season. In fact, 58% of the recorded infection in 1961 was associated with succulent terminal growth. During this year active blight was observed on terminal shoots as late as October 9. We suspect that insects have much to do with spreading the causal organism to terminal growth. Although blanket applications of parathion were made, unidentified insects were observed around the trees.

At harvest time (53 days after the last spray application) we planned to have the fruit analysed for antibiotic. Unfortunately, the analysis could not be made at that time. The fruit were therefore held in cold (33° F) storage for an additional 64 days, or 117 days after the last spray application before residue analyses could be made. At this time the fruit were assayed by the Antibiotic Division of the United States Food and Drug Administration, Department of Health, Education and Welfare. No oxytetracycline or streptomycin residue was found on or in the pear fruit.

Antibiotic sprays caused some chlorotic spotting of pear leaves, especially early in the season. As the season progressed the injury tended to disappear. If injury did not become more severe than in 1961, when eight applications were made, it is our opinion this degree of phytotoxicity could be tolerated by the trees without serious effect.

These studies suggest that epiphytotic fire blight in the field can be controlled effectively on blight-susceptible Bartlett pear provided sufficient spray applications of antibiotic are made and infected twigs are pruned out and destroyed. The second condition can easily be satisfied providing some time is spent on it. However, to satisfy the first condition a grower must decide whether he can afford the cost of the antibiotic and the time involved in making the applications required.

Because no tolerance for antibiotics on pear fruit has been set by government authorities, a third condition must be met. Antibiotics can be used only on a no-residue basis, thus restricting their use to the bloom sprays. However, under severe disease conditions it appears necessary to protect Bartlett pear trees with antibiotic to within at least 50 days of harvest. To meet this condition data would have to be gathered to prove that no antibiotic residue remains in or on the fruit if such a spray program is to be followed. Such work still must be conducted.

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RESULTS OF THREE TREATMENTS FOR THE CONTROL OF DUTCH ELM DISEASE

Lawrence R. Schreiber and James M. Harrison[1]

Abstract

Studies were conducted to determine the effect of zinc chloride, Tree Saver, and Soil Life upon incidence of infection and upon progress of Dutch elm disease in infected elms. In these tests none of the treatments either reduced disease incidence or retarded symptom expression. The first two materials caused injury to tree tissues when they were applied as recommended.

INTRODUCTION

Dutch elm disease continues to be the nation's most serious shade tree problem. Several treatments, believed by some to have merit as preventatives and cures, have attracted the public's attention through publicity in newspapers and periodicals. Use of these treatments by institutions, municipalities, and individuals has been based on reports not yet substantiated by published results of controlled experiments conducted according to commonly accepted scientific standards of design and analysis. This paper reports the results of tests with three such treatments.

METHODS AND RESULTS

Zinc Chloride: Zinc, applied as crystalline zinc chloride placed in bore holes in the tree trunk or by driving zinc nails into the tree, has been claimed to have value for control of Dutch elm disease. *In vitro* studies have shown that zinc chloride has no inhibitory effects on the growth of *Ceratocystis ulmi* (1) in concentrations up to 500 ppm. Since such a test is not always a reliable indicator of the effectiveness of a chemical in controlling plant diseases, *in vivo* studies were conducted with zinc chloride to determine its effect, if any, as a prevention of Dutch elm disease.

Fifty American elms (16.6 inches average dbh) located in an area of high disease incidence were treated. The treatment as recommended consisted of packing dry zinc chloride crystals into holes 1/2 inch in diameter and 3 inches deep bored in the tree trunk and sealing them with wooden plugs. The number of holes required for each tree equaled: $\frac{\text{diameter}^2}{36}$. Twenty-five trees received the recommended dosage while the remainder received twice the recommended dosage. The treatment was made February 1-9, 1961. Each group of treated trees was compared with an equal number of untreated trees in close proximity.

The experimental plots were observed from May through August 1961, and branch samples from all trees with disease symptoms were cultured to determine the presence or absence of *C. ulmi*. The effect of the treatments in retarding symptom expression was studied by rating trees for the percent of the crown showing foliar symptoms. Infection occurred in both treated and untreated trees in this study following natural spread.

The incidence of Dutch elm disease and severity of foliar symptoms in treated and untreated trees is compared in Table 1.

Table 1. Incidence of Dutch elm disease and percent of foliar wilt symptoms in American elms treated with zinc chloride and exposed to natural infection.

	Dosage as recommended		Dosage twice that recommended	
	treated	check	treated	check
Total number of trees	25	25	25	25
Percent developing Dutch elm disease	36.0	32.0	20.0	28.0
Average percent foliar symptoms	65.0	62.0	75.0	74.0

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[1] Plant Pathologist and Agricultural Aid, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Delaware, Ohio.

There is no indication from these data that zinc chloride retarded disease or symptom development. Phytotoxicity of the treatment resulted in reddish-brown discoloration and killing of wood and inner bark adjacent to points where the chemical was applied.

Tree Saver[2]: Tree Saver is a service furnished by Sondel, Incorporated of Mt. Vernon, Ohio, which has been publicized as being effective for Dutch elm disease control. It was tested on 499 American elms, *Ulmus americana* (approximately 11 inches average dbh), located in the Cleveland Metropolitan Park District, Cleveland, Ohio. Trees for test treatment were selected by the Park District. After they were treated, the Park District made these trees together with nearby check trees available to the United States Department of Agriculture for the comparisons reported in this section of this paper. Check trees were selected by the United States Department of Agriculture in early spring, 1961 and observations recorded from the time leaves appeared, throughout the growing season.

The service, results of which are reported here, was performed by Sondel, Incorporated in November 1960 for the City of Cleveland and consisted of two steps: 1) drilling holes 1 inch in diameter and 3 inches deep in the tree trunks 3 to 4 feet above the soil line, packing them with a formulation of chemicals, and closing the holes with wooden plugs; and 2) applying these chemicals to the soil in shallow holes around the base of the tree. Four hundred fifty-four untreated check trees were selected that were 10 to 100 yards from the respective treated trees and were similar to them in size. Neither treated nor check trees showed symptoms of Dutch elm disease when selected for this test.

The possible effect of the treatment was studied with naturally and artificially infected trees. Thirty-two of the above-mentioned treated trees and 29 untreated check trees were inoculated in mid-June with a spore suspension of *C. ulmi* through chisel cuts in the trunks. The 467 remaining treated and 425 untreated elms were exposed to natural spread of the causal fungus. The experimental plots were observed from June through September 1961 and branch samples from the crown of symptomed trees were cultured to determine whether the disease-producing organism was that of Dutch elm disease.

Treated and untreated trees were compared for the percent of the crown showing disease symptoms, and the percent of treated and untreated trees showing disease symptoms was determined (Table 2). The check trees did not show a significantly higher percentage of trees becoming infected, or greater development of the disease in infected trees than occurred in the treated trees.

The chemicals in the treatment used caused browning and killing of the inner bark and wood, frequently extending several feet above and below the points of application. The bark separated from the wood in these areas, and by the end of the growing season callus tissue began to grow at the margins of the dead wood.

Soil Life: Soil Life, an organic soil amendment developed by L. D. Gerleman and Sons, Inc., Mt. Pleasant, Iowa, was furnished for these studies by Soil Amendments Corporation, Chicago, Illinois. It was applied to soil around the roots of 50 American elms 1/2 to 5/8 inch

Table 2. Incidence of Dutch elm disease and percent of foliar wilt symptoms in American elms treated with Tree Saver versus untreated trees, both naturally and artificially infected.

	Naturally infected trees		Artificially infected trees	
	treated	check	treated	check
Total number of trees	467	425	32	29
Percent developing Dutch elm disease	11.9	2.1	81.3	82.8
Average percent foliar symptoms	67.5	68.4	80.3	73.2

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[2] Acknowledgment is given to the Holden Arboretum, Mentor, Ohio and the Cleveland Metropolitan Park District, Cleveland, Ohio for their cooperation in this research.

caliper in our experimental nursery at the time of planting in April 1960. The initial application was made at the rate of 1 pound/inch caliper. In July and November 1960 additional Soil Life was applied at the rate of 2 pounds/inch caliper to the soil surface around each tree and mixed with the soil by cultivation and watering. The treated trees and 50 untreated check trees were inoculated with a spore suspension of C. ulmi in May 1961 through knife wounds in their trunks. All treated and untreated trees developed symptoms of Dutch elm disease in about 4 weeks and died. These findings corroborate those of Neely and Himelick (2) in Illinois and Smalley (3) in Wisconsin that Soil Life has no measurable effect on the incidence or severity of Dutch elm disease.

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INFLUENCE OF GIBBERELLIN ON BUD DIFFERENTIATION OF SOUR CHERRY AND ON DAMAGE BY SOUR CHERRY YELLOWS[1]

Wladyslaw Basak, K. G. Parker, R. W. Goodno, and T. H. Barksdale[2]

Abstract

Gibberellin, applied as potassium gibberellate to sour cherry trees affected by sour cherry yellows, induced the development of vegetative buds on the terminals. The percentage of vegetative buds varied with the dosage applied. The addition of glycerol or Tween 20 to the mixture enhanced the effect. This treatment offers promise as a means of preventing excessive blossom bud formation and consequent yield reduction associated with this disease.

Sour cherry yellows is generally considered the most important virus disease of sour cherry in northeastern United States. One of the significant damaging effects is the production of more than the normal number of flower buds on the terminals at the expense of vegetative buds. Eventually the fruit spur system of the tree is significantly reduced and yields are much less than those on trees not affected by this disease (3). It is generally considered, and a common observation, that growth of the trees also is reduced.

Hull and Klos (2) found that gibberellin treatment induced more prolonged and more total growth on yellows-infected trees, and also on trees containing ring spot virus, as well as on virus-free trees. These tests were made on small potted trees. It seemed worth while to test this treatment under orchard conditions to determine whether the growth of mature yellows-infected trees might be improved.

EXPERIMENTAL

1959: Potassium gibberellate spray was applied in two mature orchards in which all or nearly all trees were infected with yellows and in one younger bearing orchard in which only part of the trees were infected. The first spray was applied approximately 1 week after petal fall; on different rows or parts of rows single applications were made, or two were made 1 week apart. Where only one application was made, it was made in one experiment at the early date and in another at a later date. In all cases the concentration was 100 ppm gibberellin.

As expected, terminals continued growth later in the season in all treatments and slight twisting of the leaves was observed. The striking effect, however, was the almost complete absence of fruit buds on the terminals, with a vegetative bud produced at almost every node. A very heavy production of fruit spurs occurred on the treated trees in 1960 (Figs. 1 and 2). The buds on these spurs were strong and examination in late winter of 1960-1961 indicated that less low temperature injury occurred in buds on yellows-infected trees that were treated than on comparable untreated trees.

The treated trees produced a heavy bloom in 1961 and set a very heavy crop of fruit. In one experiment, where a single row of trees was sprayed twice in 1959, the yield in 1961 on the treated row averaged 222 pounds per tree, compared with 124 pounds per tree in the remainder of the orchard. If the 1960 yield is added to the latter figure the total yield for 2 years was 222 on the treated compared with 208 on the untreated trees. Soluble solids content of fruit in 1961 from treated trees was 11.8% compared with 12.3% on untreated trees, a reduction probably caused by the low leaf/fruit ratio. Weight of fruit in 1961 was 17 ounces per 100 fruits on treated compared with 19.5 ounces on untreated trees.

Similar differences were obtained in the experiments on two other farms. In these cases one or two applications made in 1959 had about the same result and it appeared to make little difference whether the one application was made at the early or the late date. In one of these

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[1] Alan L. Jones and R. R. Fisher assisted in the field work. The Sodus Fruit Farm, where part of the work was done, made the spray applications with the air-blast sprayer. Gibberellin materials were supplied gratis by Merck and Co., as Gibrel, 0.5% or 2.5% potassium gibberellate, and the ester form, 4% ethylene glycol monobutyl ether gibberellate.

These investigations were supported in part by federal funds, both Hatch Amended and Regional Research.

[2] T. H. Barksdale's present address: Crops Division, Fort Detrick, Frederick, Maryland.

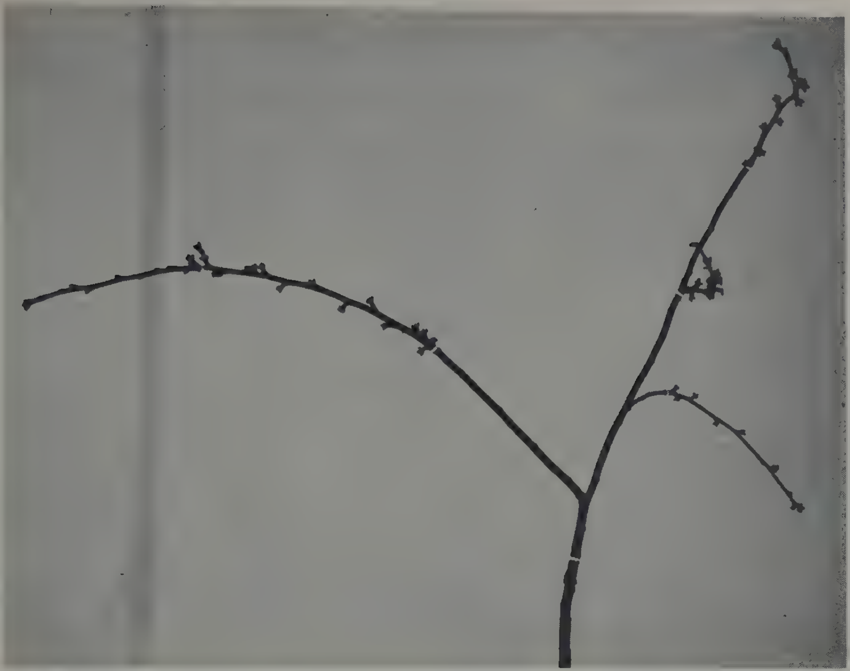

FIGURE 1. Montmorency twig from tree sprayed with gibberellin in 1959. Photographed November 1960. White tape marks bud scale scars at base of shoot growth in successive years. Note abundant fruit spurs on 1959 shoot growth.

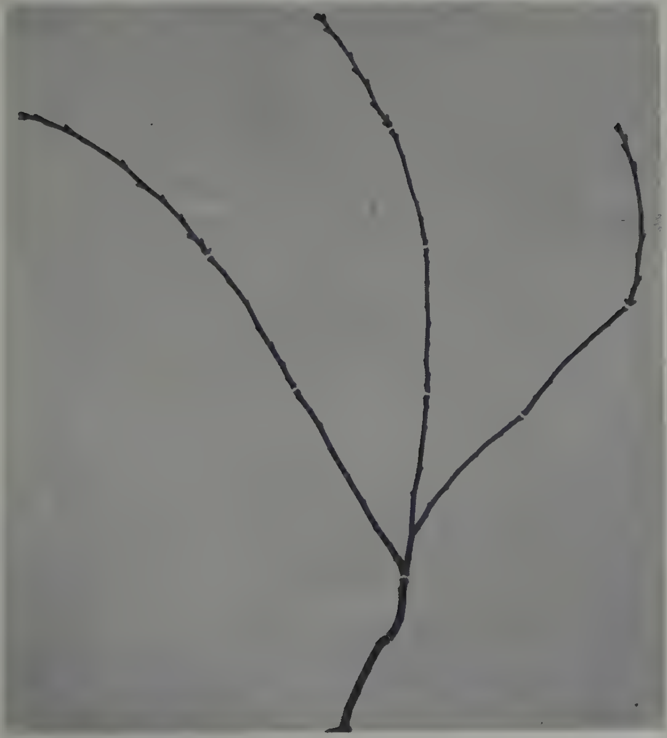

FIGURE 2. Montmorency twig from untreated tree. Photographed November 1960. White tape marks bud scale scars as in Figure 1. Note complete absence of fruit spurs, and no lateral shoots in last 2 years.

latter experiments part of the trees were not affected by sour cherry yellows and the effect on these trees was similar with respect to bud differentiation in 1959 to that on the trees infected by yellows. General observation indicated that on the yellows-free trees the yield in 1961 was so heavy that the fruit failed to mature properly. This is one hazard that would have to be taken into account should this treatment be used in a practical way.

1960: Because the prevention of fruit bud formation in 1959 was so nearly complete, experiments were made with single applications of a series of concentrations in 1960, beginning at 50 ppm and progressing downward. Timing was similar to the first application in 1959. Little observable effect occurred. Bud examination in the following winter indicated that on treated trees less than 10 lateral buds in 100 produced were leaf buds in the two experiments conducted in 1960, with no leaf buds on the untreated trees. The result was similar on yellows-infected and on yellows-free trees. No explanation for this failure is yet available, pending further study of the conditions in the 2 years and the results of additional experiments.

1961: More extensive experiments were made to compare dosages, timing, the incorporation of adjuvants in the spray mixture, and to compare the acid form with the ester form of

Table 1. Bud differentiation on Montmorency sour cherry trees sprayed with gibberellin. Cohn Farm, Sodus, New York. 1961

Treatment number	Concentration (ppm)	Date sprayed 1961	% vegetative buds[a]
1	50	June 9	57.4
2	75	June 9	69.6
3	25	June 14	12.4
4	50	June 14	42.8
5	75	June 14	39.5
6	100	June 14	70.6
7	50 + G[b]	June 14	60.7
8	75 + G	June 14	67.4
9	50 + T[c]	June 14	64.6
10	75 + T	June 14	65.3
11	50 E[d]	June 14	39.6
12	100 E	June 14	50.9
14	50	June 19	43.8
15	75	June 19	72.0
13	none	---	4.6
16	none	---	4.9

[a] Each figure the average of two trees, with buds on 10 terminals examined on each tree, the tip bud on each terminal excluded.

[b] Glycerol added at 2 quarts in 100 gallons of spray.

[c] Tween 20 added at 1 pint in 100 gallons of spray.

[d] Ester form of gibberellin. All other treatments made with the acid form.

Table 2. Bud differentiation on Montmorency sour cherry trees sprayed with gibberellin. Combinations of treatments to make special comparisons. Cohn Farm, Sodus, New York. 1961

Factor	Treatment number	Treatment identity	Concentration[a] (ppm)	% vegetative buds
Timing	1 + 2	early	50 + 75	63.5
	4 + 5	middle	50 + 75	41.2
	14 + 15	late	50 + 75	57.9
Compound	4 + 6	acid	50 + 100	56.7
	11 + 12	ester	50 + 100	45.3
Adjuvants	4 + 5	none	50 + 75	41.2
	7 + 8	glycerol	50 + 75	64.1
	9 + 10	Tween 20	50 + 75	65.0
Dosage	1, 4, + 14	all dates	50	48.0
	2, 5, + 15	all dates	75	60.4
Check	13 + 16	--	--	4.8

[a] Concentrations listed within any given grouping are the same on each line of the group. Combinations of treatments in this table were made to increase the number of treated trees for each comparison.

Table 3. Bud differentiation on Montmorency sour cherry trees sprayed with gibberellin. Sodus Fruit Farm. 1961

Dosage	% vegetative buds[a]
Gun Spray	
25 ppm	10.6
50 ppm	31.0
75 ppm	44.0
100 ppm	47.5
none	1.5
Air-blast	
50 ppm - 50 gal./acre	15.8
50 ppm - 100 gal./acre	15.4
50 ppm - 200 gal./acre	29.9
none	3.1

[a] Each figure the average of determinations on four trees, 20 terminals on each tree, the tip bud on each terminal excluded.

gibberellin. An additional test consisted of a single concentration of gibberellin, 50 ppm, applied with the air-blast sprayer at reduced gallonages. Single applications were made.

In the experiment represented by data presented in Tables 1 and 2, the first application was made about 1 week after petal fall, as in the previous studies. Two trees were sprayed with each treatment and all were sour cherry yellows-infected trees. In October 1961, buds were examined on 10 terminals collected from each tree at random at a convenient height from the ground. Dates of applications are of doubtful significance because applications made at the same concentrations on the first and third dates gave more effect than did those at the intermediate date. The addition of either glycerol or Tween 20 markedly enhanced the effect of gibberellin. The acid form of gibberellin was more effective than was the ester form at the same concentrations.

Table 3 presents data from another orchard where two experiments were conducted. All trees were mature and all were infected with sour cherry yellows. In one experiment in this orchard four trees were treated with each of four different concentrations of gibberellin compared with four untreated trees. In the same orchard, but in a different area, the test was made with 50 ppm applied with the air-blast sprayer at different gallonages per acre. Buds were examined in January 1962, from 20 terminals collected from each tree.

The data presented in Table 3 indicate results similar to those in the experiment represented in Tables 1 and 2, but the effects appear to be somewhat less. This may be because the yellows disease in this orchard is somewhat more severe than is that in the first orchard. The concentration series test was made with the conventional spray gun. The gallonage test was made with an air-blast sprayer, and the experiment was designed for the highest dosage applied with the air-blast sprayer to approximate half that applied with the gun, which was approximately 400 gallons per acre. Four trees in a single row sprayed with each dosage of the air-blast were examined. The data indicate that the result with 200 gallons at 50 ppm was about the same with the air-blast as on the trees sprayed with 50 ppm with the gun, using approximately 400 gallons per acre, or twice that applied with the air-blast sprayer. These data, of course, are not conclusive but there is an indication that with the air-blast providing reasonably even distribution of spray over the tree without so much runoff as is experienced with the gun spray the effect may be greater in proportion to the number of gallons applied. It is perhaps significant to note that the percentage of blossom buds produced on these trees when untreated is higher in the lower part than in the upper part of the tree. For this reason the type of application with the air-blast sprayer, which usually delivers more spray in the lower part of the tree, may be favorable for this treatment, which is needed more in the lower part than in the upper part of the tree.

DISCUSSION

Because of the very pronounced effect of gibberellin sprays on bud differentiation on terminals, following the experience in 1959 the study has been primarily directed toward this factor. Bradley and Crane (1) reported testing the effect of gibberellin on bud development in several species of Prunus, including cherry. They suggest the influence is on bud initiation rather than differentiation. They found that with a little over 200 ppm applied at full bloom, flower bud development was prevented, and at twice this concentration vegetative bud initiation as well was prevented. They indicated, and our experience tends to confirm this, that the effect is all or none. That is, in our experience no weak flower buds were produced. There was either a normal flower bud at any given node or there was a vegetative bud. It seems, therefore, that it should be possible to find a dosage of gibberellin which when applied to a tree infected with yellows will provide bud differentiation on the terminals similar to that which normally occurs on yellows-free trees. In this way the current crop might be maintained at a reasonably satisfactory level and still provide for enough vegetative buds to make new shoots and fruiting spurs for subsequent crops in the succeeding years. The data in Tables 1 and 2 seem to indicate that the dosage may not be so critical but that this can be made practical. Timing appears to be important, but not necessarily very critical with respect to growth stage of the tree. Weather conditions at the time of application remain to be studied. The addition of adjuvants such as glycerol and Tween 20, tried in these experiments, appears to offer promise at least for reducing the amount of gibberellin needed and thereby reducing the cost.

It is suggested that the greatest benefit expected is on trees younger than those used in most of these experiments, although possibly fully mature trees severely affected by yellows virus may be brought back into profitable production by heavy pruning followed by treatment with gibberellin to prevent them from returning to their present poor productive state. Observations in recent years indicate that substantial improvement might be achieved by heavy pruning alone every year. Moderately affected trees will respond to heavy pruning and produce many fruit spurs on 2-year-old shoots, but these shoots will soon revert to the usual barren condition associated with yellows virus disease.

There is no indication that this treatment has any direct effect on the yellows virus itself. In fact, the treated trees apparently develop yellows symptoms to about the same degree as do the untreated trees. Present studies on this factor indicate that the effect is not on the virus but rather on counteracting the influence of the viruses on bud differentiation. There is no indication that this treatment could in any way take the place of planting virus-free trees in isolated locations to reduce rate of spread of the virus. But there is an indication that this treatment might help to maintain infected trees in a profitable level of production for a longer time than is experienced with the average yellows-infected tree.

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STUDIES ON THE HOST RANGE OF WATERMELON MOSAIC VIRUS IN HAWAII

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Abstract

Of 29 species of plants representing 14 families common to Hawaii, only those in Cucurbitaceae were found susceptible to watermelon mosaic virus, when tested by sap inoculations and by aphid transmissions using *Myzus persicae* (Sulzer). *Momordica balsamina*, the most common wild cucurbit in Hawaii, and the only wild one tested, is probably the wild reservoir from which aphids transmit the virus to watermelon and other cultivated cucurbits.

INTRODUCTION

Walker (3), who first reported on the watermelon mosaic, suspected that a few of the wild plants he observed could serve as hosts for the watermelon mosaic virus, but did not test his hypothesis. Anderson (1) tested 13 species of cucurbits by sap inoculations and found all but one susceptible to the virus. He also tested three noncucurbits and found them not susceptible. Grogan, Hall, and Kimble (2) mechanically inoculated 35 species of plants representing seven families not including Cucurbitaceae, and found some members of Umbelliferae, Malvaceae, and Leguminosae susceptible to the virus.

Successful control of aphid-borne virus diseases depends upon elimination of either the virus host plant, the vector, or both. In some cases, elimination of the host plant is more feasible than control of the vector. It is important, therefore, to know which plants are able to serve as hosts.

This study was conducted to determine what species of plants common in Hawaii can serve as hosts to the watermelon mosaic virus. Both aphid and sap transmissions were made because of the possibility that the virus can be transferred by one method and not the other.

MATERIALS AND METHODS

The susceptibility to watermelon mosaic virus of various species of plants representing the common plant families in Hawaii was tested. Fasted green peach aphids, *Myzus persicae* (Sulzer), were transferred singly to test plants after an acquisition feeding of 15 to 20 seconds. Watermelon plants which were sap inoculated 10 to 14 days earlier served as virus-source plants. Twenty-five test plants were used for aphid transmissions and 10 plants for sap inoculations. Only sap inoculations were made for some plant species where insufficient number of plants were available.

Two weeks after inoculation of the host plants, back inoculations to watermelon seedlings were made; 25 plants inoculated by aphids and 10 by sap inoculations. Back inoculations were made in every case because some plants may have been symptomless carriers, and also because of the possibility that a plant may harbor the virus and yet not be a source for subsequent transmissions. The plants used for back inoculations were selected on the basis of suspected symptoms as compared with control plants.

Sap inoculations were made by lightly rubbing sap extracts together with carborundum on the leaves of the test plants. Both sap extracts and carborundum were then washed off with water.

RESULTS AND DISCUSSION

Plants which were apparently neither susceptible nor symptomless carriers, based on the lack of infections by either aphid transmission or by sap inoculation, or both, belong to the following species in their respective families:

CARICACEAE--*Carica papaya*, papaya. CHENOPODIACEAE--*Chenopodium amaranticolor*, American wormseed. COMPOSITAE--*Bidens pilosa*, Spanish needle; *Emilia sonchifolia*, Floras paintbrush; *Sonchus oleraceus*, sow thistle; *Xanthium pensylvanicum*, cocklebur. CONVOLVULACEAE--*Ipomoea cairica*, morning glory. CRUCIFERAE--*Brassica* juncea, kai-choi, mustard. EUPHORBIACEAE--Euphorbia geniculata, wild euphorbia; Ricinus communis, castorbean. LABIATAE--Leonotis nepetaefolia, lions-ear. LEGUMINOSAE--Crotalaria mucronata, rattlebox; Desmodium uncinatum, Spanish clover; Indigofera suffruticosa, indigo; Leucaena glauca, koa haole, or white popinac; Phaseolus vulgaris var. humilis, bush bean. MALVACEAE--Hibiscus esculentus, okra. NYCTAGINACEAE--Mirabilis jalapa, four-o'clock. PHYTOLACCACEAE--Phytolacca octandra, southern pokeberry. SAPINDACEAE--Cardiospermum halicacabum, balloon vine. SOLANACEAE--Datura stramonium, Jimsonweed; Nicotiana glutinosa, tobacco; Solanum nigrum, black nightshade, or popolo.

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Plants belonging to the species in the family Cucurbitaceae which were tested for susceptibility to watermelon mosaic virus were: Cucumis melo, honeydew melon; Cucumis melo var. cantalupensis, cantaloupe; Cucumis sativus, cucumber (Colorado Long); Cucurbita maxima, autumn and winter squash (Hubbard squash); Cucurbita pepo, field pumpkin; Momordica balsamina, balsam-apple. All of these species were found to be subject to both sap and aphid transmission of the virus, including back inoculations.

The apparent failure to transmit watermelon mosaic virus to plants other than cucurbits in Hawaii points to the likelihood that the virus is harbored in cultivated and wild cucurbits. In areas where watermelons are grown, other cucurbits such as cucumber, squash, and pumpkin are also grown, thereby possibly maintaining a constant source of the virus from which aphids can transmit to watermelon. Momordica balsamina is the commonest wild cucurbit in Hawaii, and it undoubtedly serves as a good source from which aphids can transmit the virus to cultivated cucurbits.

Although this study did not show that plants in families other than Cucurbitaceae are susceptible to watermelon mosaic virus, there may be plants which are susceptible, as demonstrated by Grogan, et al. (2). Therefore, if more species of plants are tested, it is probable that some species will be found to be able to serve as a reservoir in the field. Findings from such studies can then be applied in preventive and control measures against the spread of the virus.

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NEW PHYSIOLOGIC RACES OF BEAN RUST, UROMYCES PHASEOLI TYPICA, FROM MEXICO

Alfonso Crispin and Segundo Dongo[1]

Summary

Thirty-one physiologic races of bean rust are reported from Mexico and the levels of infection previously used in indentification studies have been re-grouped into a scale which ranges from 0 to 5.

Rust is an important disease of beans in the bean-producing areas of Mexico. Because of their susceptibility to this disease, some bean varieties are no longer being planted along the Pacific Coast (States of Nayarit and Sinaloa) and in the northern parts of the country. Since 1955 several resistant bean varieties (Bayomex, Canario 101, Canocel and Mecentral) have been released; in recent years however, Canario 101 has been observed to be slightly infected. This change in host reaction could be the result of a possible development of new races of the fungus. For this reason, a research project was planned to study the race complex and its geographic distribution. The results obtained from these studies are presented and briefly described in this paper.

Race Complex: In Mexico, Crispin (1) made the first monographic study of the disease and attempted to differentiate the variants of the rust organisms. Preliminary results showed the presence of 16 races, different from the 20 reported by Harter and Zaumeyer (3). He therefore suggested that a group of Mexican bean varieties be utilized as differentials since those used in the United States were susceptible to most of his isolates. Fisher (2) recently summarized the work on the identification of the races of *Uromyces phaseoli typica* Arth. in the United States. This compilation shows that from 1941 to 1951 the number of races identified amounted to 30. In 1954 Sappenfield (4) reported race 31 from New Mexico and Zaumeyer (5) the race 32 from Maryland.

How the Work Has Been Conducted: The collection of diseased plant material has been made throughout the main bean-growing areas. Purification of inoculum and the inoculations were made according to the procedure described by Harter and Zaumeyer (3). The scale used by these authors has been modified in that the ten levels of infection reported have been grouped into only five, which can be clearly separated, according to the following system (Fig. 1):

0 - Without symptoms, immune.
1 - Small necrotic lesions, no pustules.
2 - Numerous small pustules surrounded by a necrotic area.
3 - Numerous small pustules, hardly visible on the lower leaf surface; no necrosis.
4 - Numerous pustules of fairly good size are distributed all over the upper and lower leaf surface; sometimes a chlorotic halo is observed around the pustules.
5 - Numerous large pustules distributed on the upper and lower leaf surface, margins of leaves are dead or the entire surface becomes chlorotic.

Table 1. Differential varieties and their reaction to the 31 physiologic races of bean rust (*Uromyces phaseoli typica*) which have been identified in Mexico.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Aguascalientes 13	4	5	5	5	1	1	5	1	4	4	1	1	4	5	5	5	5	5	5	5	5	5	4	5	5	5	2	5	5	5	5
Guerrero 6	4	4	0	3	4	4	4	0	1	1	4	4	4	0	0	0	5	5	0	4	5	0	0	4	0	4-5	2	0	3	4	3
Guerrero 9	4	0	5	4	0	4	5	4	0	5	0	4	4	4	4	0	4	5	3	1-3	2	1-3	1	3-4	5	5	5	5	5	3	3
Guanajuato 10A-5	0	5	0	0	4	4	5	4	4	4	4	4	3	0	4	0	4	5	4	4	4-5	5	5	5	5	5	0	5	5	5	4
México 6	4	5	4	0	5	4	3	1	3	4	3	0	4	4	4	5	3-4	5	3	2-3	4	3	5	1	4	4	1	4-5	1	3	3
México 12	4	0	0	3	4	4	5	4	5	4	4	4	5	4	4	4	5	5	5	5	5	5	5	5	5	5	3	5	4-5	5	5
Veracruz 10	0	0	0	3	4	4	5	4	0	4	4	5	5	0	5	0	0	5	0	5	0	0	0	0	5	0	5	0	5	0	5
Canario 101	0	1	0	1	4	1	0	0	1	1	0	1	1	1	0	1	1	1	1	1	4	3	1-3	1	3-4	1	1	3-4	1	4	3
Negro 150																	5	5	5	0	5	4-5	0	5	5	0	2	5	4	0	5

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[1] Respectively, Plant Pathologist in charge of the bean improvement program of the Instituto Nacional de Investigaciones Agricolas, S. A. G., Mexico and former research scholar - The Rockefeller Foundation.

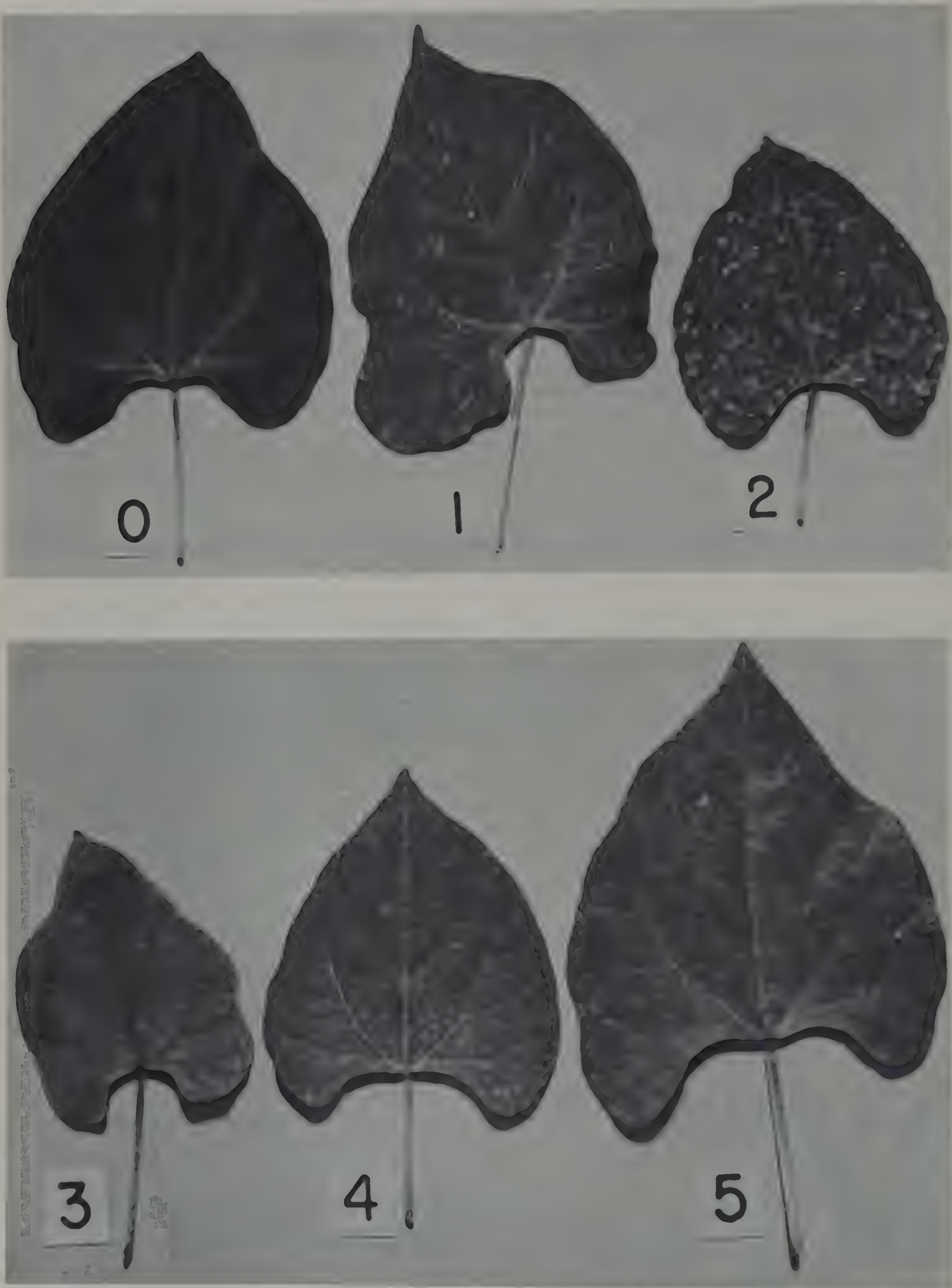

FIGURE 1. Typical infection types produced by the bean rust organism on a group of differential bean varieties.

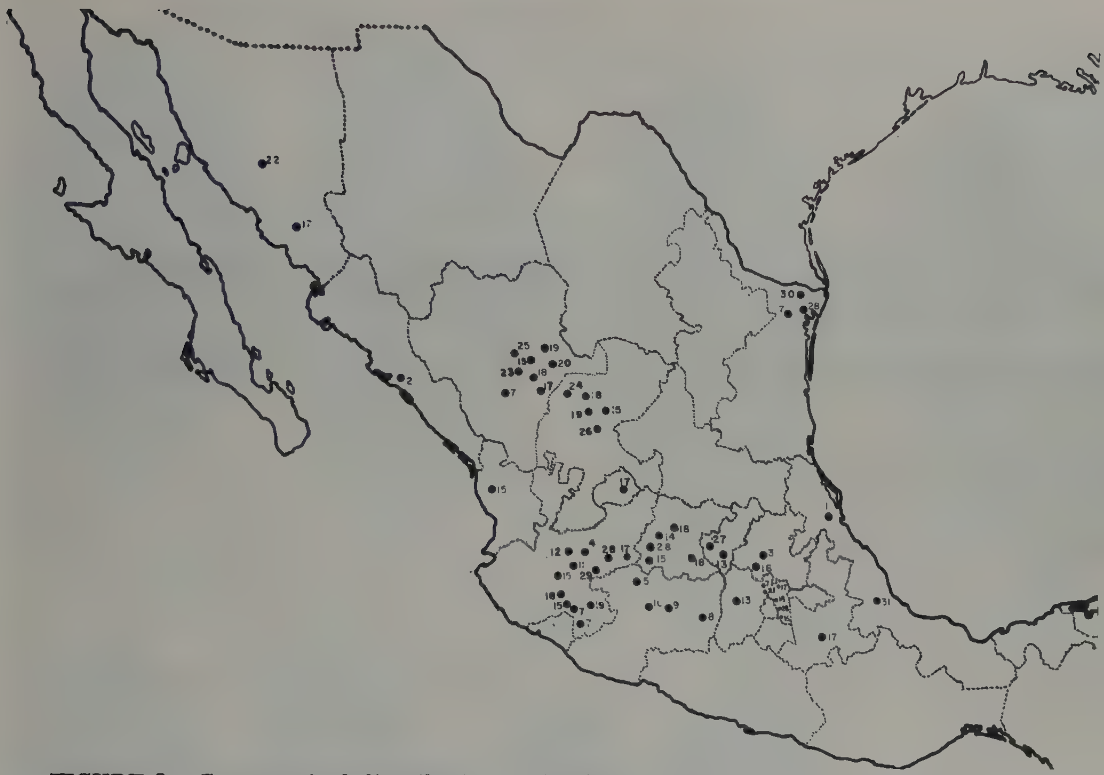

FIGURE 2. Geographical distribution of the thirty-one physiologic races of bean rust which have been identified in Mexico.

In order to compare the Mexican races with those already identified in the United States, the two sets of differential varieties were inoculated (1, 3).

Experimental Results and Conclusions: After about 200 collections of diseased material had been screened, 15 new races (Nos. 17 to 31), all different from those reported to date in the United States, were separated by their reaction on a group of Mexican bean varieties. Total known races in Mexico now number 31 (Table 1).

The variety Canario 101 is very resistant to almost all races of bean rust. Since it is also an early maturing variety of good quality and desirable bush type, it has been used extensively as a parent in the breeding program and is the variety most widely distributed in the country. Its susceptibility to some races (Table 1) has caused bean breeders to look for some other source of resistance in the bean germplasm, and to determine the reaction of the advanced hybrid lines and commercial varieties to the new races. Recommendations would then be made according to the varietal reaction and the geographical distribution of such races.

From these studies several conclusions can be made: 1) A modification to the grades of infection previously used (3) is proposed. 2) Fifteen new races were isolated from the main bean-producing areas of Mexico (Fig. 2). 3) The appearance of new races, as demonstrated by greenhouse studies and confirmed by field observations, emphasizes the need for a reevaluation of the bean germplasm used in the bean breeding program.

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CUCUMBER, AN ADDITIONAL HERBACEOUS HOST OF THE INFECTIOUS VARIEGATION STRAIN OF CITRUS PSOROSIS VIRUS[1]

Paul R. Desjardins and James M. Wallace[2]

Abstract

The infectious variegation strain of citrus psorosis virus has been transmitted to the "Chicago Pickling" variety of cucumber, Cucumis sativus. Symptoms on the inoculated cucumber cotyledons consisted of diffuse chlorotic rings and chlorotic spots. The virus was transmitted from the inoculated cucumber cotyledons to Cascade Lemon, cucumber and Crotalaria spectabilis. Recovery of the virus from uninoculated parts of the cucumber plant was not accomplished. Cool temperatures favored the infection of cucumber with the virus.

INTRODUCTION

The infectious variegation strain of the citrus psorosis virus produces stronger symptoms on leaves of lemon and sour orange than other strains of the virus (7). These symptoms were first described and considered to be a form of psorosis by Fawcett and Klotz (2).

Grant and Corbett (3, 4) recently reported mechanical transmission of the infectious variegation virus from citrus to citrus and from citrus to two noncitrus hosts, namely cowpea (Vigna sinensis) and Crotalaria spectabilis. Although cucumber, Cucumis sativus, was included in their tests these authors did not find it to be susceptible to infection.

This paper reports successful transmission of infectious variegation to the "Chicago Pickling" variety of cucumber. The variety tested by Grant and Corbett (4) was Marketer.

METHODS AND RESULTS

The virus was cultured in lemon, sour orange, and grapefruit. The source of the virus traced directly back to the original isolate described by Fawcett and Klotz (2).

In the initial experiments the inoculum was prepared by grinding soft, young, virus-infected citrus leaves in liquid nitrogen in a mortar. The frozen powder was suspended in pH 8 buffer (0.02M PO_4). The resulting suspension was further homogenized in a mechanical blender and passed through cheese cloth in a hydraulic press. The extract was then rubbed on the cotyledons of very young cucumber plants that had been dusted with carborundum powder. The plants were rinsed with water shortly after inoculation. When this method was used the percentage of infection ranged from 10 to 20%. When 0.01M sodium diethyl dithiocarbamate and 1% thioglycolic acid were included in the buffer, infections sometimes were as high as 80%.

FIGURE 1. Cucumber cotyledons showing the diffuse chlorotic rings. Mechanically treated check cotyledon on the right.

FIGURE 2. Mechanically treated check cotyledon on the left. The three cotyledons on the right show the chlorotic spots caused by the virus.

FIGURE 3. Virus-infected cucumber cotyledon after clearing with alcohol and staining with iodine solution.

FIGURE 4. Cascade lemon seedling plant infected with the virus from cucumber.

FIGURE 5. Crotalaria spectabilis plants inoculated with the virus from cucumber. The two plants in the foreground have been killed by the virus.

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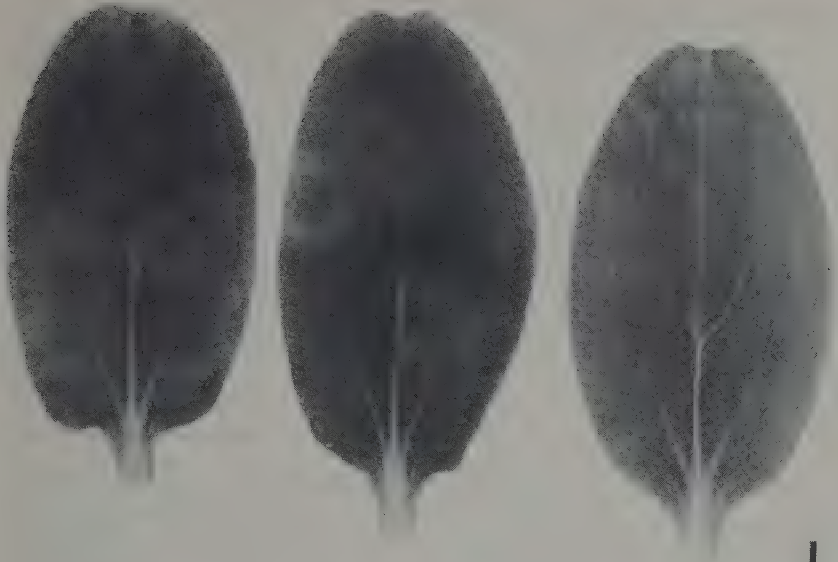

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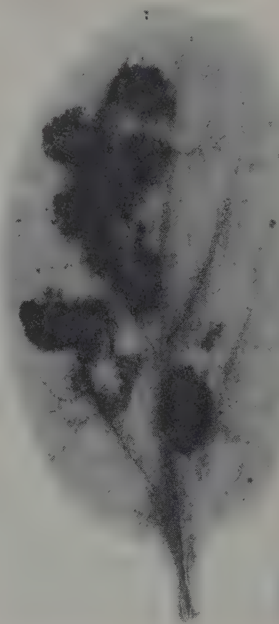

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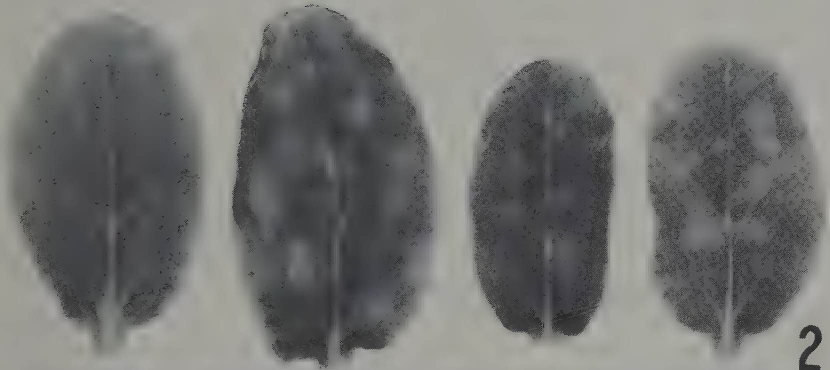

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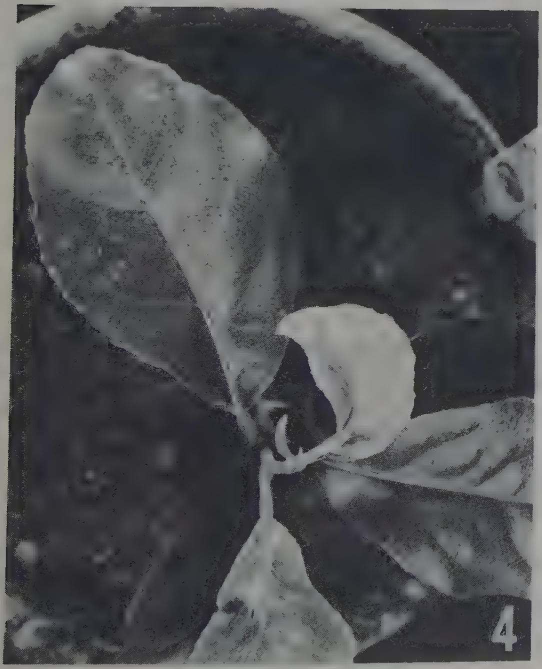

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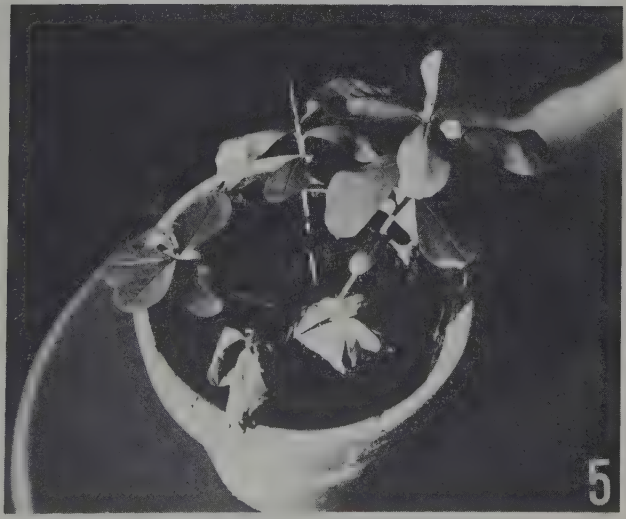

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In later experiments the virus inoculum was prepared as described by Grant and Corbett (3, 4). Soft, young leaves from infected citrus plants were minced in a mortar containing 20% sucrose and charcoal. The resulting preparation was frozen for 2 to 6 hours, thawed, ground and extracted. These extracts usually gave a higher percentage of infection of cucumber plants than the extracts prepared by the first method described. However, high percentages of infection were obtained most consistently when the inoculum was prepared as described below.

Two to 4 grams of virus-infected citrus leaves were ground in liquid nitrogen in a mortar. The frozen powder was suspended in 10% sucrose solution without charcoal. The suspension was thawed but kept in an ice-water bath until inoculations were completed. This method of inoculum preparation has on occasion resulted in 98 to 100% infection of cucumber plants.

Symptoms consisted of diffuse chlorotic rings or more or less distinct chlorotic spots, which developed on the inoculated cotyledons within 7 to 14 days (Figs. 1 and 2). When the symptoms were first noted on cucumber, Bawden's modification (1) of Holmes' starch-lesion method (5) was employed to accentuate the lesions. Figure 3 illustrates the type of starch lesion obtained by this technique.

Rarely were both rings and spots observed in the same experiment. The cause of the variation in symptoms is unknown, but temperature and day length (time of year) seemed to have some effect.

Grant and Corbett (4) demonstrated that more infection of lemon and Crotalaria was obtained by holding plants at a low temperature after inoculation. In our mechanical transmission studies with cucumber as a host of the citrus infectious variegation virus, the local lesion symptoms described herein have developed more regularly and more conspicuously in experiments conducted during the winter months as compared with the times of the year when greenhouse temperatures are high.

The virus has been transmitted from lesions on cucumber to cucumber, Cascade variety of lemon (Fig. 4) and Crotalaria spectabilis (Fig. 5). Although some virus-like symptoms occasionally developed on the true leaves of inoculated cucumber plants, we have been unable to recover the virus from uninoculated parts of the plant.

DISCUSSION

Although the "Chicago Pickling" variety of cucumber is obviously not a suitable host for culturing the infectious variegation virus, the results here reported suggest that it might show promise as an assay plant for the virus in at least a qualitative sense.

Holmes (6), reporting on his studies of the transmission of potato mottle virus to and from citrus, suggested that there are no virus inhibitors in citrus foliage. This has not been contradicted, but our findings support the suggestion of Grant and Corbett (4) that sucrose increases the infectivity of the inoculum. We have obtained higher infection percentages when sucrose solutions were employed in extraction procedures than when it was not used. The use of charcoal in extraction, however, does not appear to be essential for transmission of the virus.

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EFFECT OF REMOVAL OF EGGS FROM CYSTS ON HATCHING OF LARVAE OF HETERODERA SCHACHTII

Arnold E. Steele[1]

The factors affecting hatching of eggs of cyst nematodes of the genus *Heterodera* are of considerable inportance in conducting hatching experiments. Although it has often been suggested that availability of oxygen to the eggs in the cyst is important, no clear evidence for or against has been presented.

Wallace (2) demonstrated that emergence of larvae from cysts of *Heterodera schachtii* Schmidt, 1871 was decreased by exposure to low oxygen concentrations for short times. Onions (1), working with cysts of *Heterodera rostochiensis*, found evidence which led him to believe that "although the cyst wall is permeable to the hatching stimulant, it is argued that the hatching pattern is more likely due to a gradient of oxygen tension within the cyst." Wallace (3) found that the rate of hatching increases with aeration of the soil environment.

One method of increasing availability of oxygen to the eggs is by removing them from the cyst. The hatching of larvae from excised eggs can then be compared with hatching from eggs remaining in the cyst. Apparently this had not been tried.

A collection of *Heterodera schachtii* cysts were divided into three groups, each consisting of eight replications of 20 cysts. Each of two cyst groups were placed in Syracuse watch glasses containing tap water or beet root diffusate, and allowed to remain for 3 weeks. At the end of the treatment period, the emerged larvae were counted and the cysts were broken open so that hatched larvae which had not emerged from the cysts could be counted. Eggs and larvae were removed from cysts of a third group and the empty cysts discarded. Hatched larvae were counted and the eggs placed in tap water for 3 weeks. Larval counts appear in Table 1.

Table 1. Effect of removal of eggs from cysts on hatching of larvae of *Heterodera schachtii*.

Treatment	Total[a]	Av. no. larvae/cyst
1. Larvae hatched from eggs in tap water	2,427	15.2
Larvae removed from cysts	3,577	22.3
Total	6,004	37.5
2. Larvae emerged from cysts in tap water	5,080	31.7
Larvae left in cysts after treatment	462	2.9
Total	5,542	34.6
3. Larvae emerged from cysts in beet diffusate	32,539	203.4
Larvae left in cysts after treatment	161	1.0
Total	32,700	204.4

[a] Total number of larvae in eight replications.

Since removal of eggs from cysts resulted in an average increase in hatch in tap water of only about three larvae per cyst, this treatment did not appear to influence hatching appreciably. This conclusion is supported by the observation that more than five times as many larvae emerged from intact cysts in beet diffusate as from either the intact cysts or free eggs in tap water.

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SCLEROTIUM ROLFSII IN ISRAEL[1]

Zehara Avizohar-Hershenzon and J. Palti[2]

Abstract

A list is presented of 50 hosts of Sclerotium rolfsii in Israel. Eight hosts are believed to represent new records for this fungus. Among isolates from 11 hosts, only that from violets constituted a distinct strain.

In Israel S. rolfsii affects only irrigated spring-summer crops. Rapid drying of the surface soil appears to prevent infection of widely spaced plants in their early stages of growth. Infection develops chiefly when more fully grown plants shade the soil around their collars.

Israeli studies on the following aspects of S. rolfsii research are summarized: physiology, especially as affected by growth-regulating substances; control effects of nitrogen; control by non-tillage practices in apple orchards; and resistance of apple stocks.

INTRODUCTION Sclerotium rolfsii Sacc. is one of the most important plant pathogens in Israel and has been investigated for the past 30 years. Unfortunately, the few published reports are in Israeli journals, not readily accessible abroad. This paper summarizes this information as well as hitherto unpublished studies on this fungus and its development under the arid conditions of Israel.

The Symposium on Sclerotium rolfsii (2) provides an invaluable background for further publications on this organism. Therefore in the present report an attempt is made to emphasize results which differ from or supplement those presented in the Symposium.

HOST RANGE Fifty hosts on which S. rolfsii has been recorded in Israel are given in the host list. This includes hosts mentioned by Reichert (18, 19) and records since made by the staff of the Division of Plant Pathology at Beit Dagan and by pathologists of the Department of Phytopathology of the Plant Protection Service, Jaffa; eight of these hosts are believed to be new reports: Amaryllis sp., Calla sp., Cydonia vulgaris, Cynodon transvaalensis, Olea europaea, Trifolium alexandrinum, Tritonia sp., and Valeriana italica. The records on olive (see host list) are interesting, as this host was previously considered immune (1). Our list also includes records on Vitis vinifera on which S. rolfsii has so far been found in Formosa only (20).

PHYSIOLOGICAL STUDIES Physiological studies with S. rolfsii isolates from apples, and cultured mostly on potato-dextrose agar or broth, led Lavee (6) to the following conclusions: 1) Secretions. The mycelium, even when sclerotia are germinated on filter paper or on glass-wool (containing no cellulose supply), invariably secretes oxalic acid; this is to be considered part of its basic metabolism. 2) pH values. At the beginning of mycelial development in cultures, the pH of the nutrient drops rapidly, then remains low for a short period before rising again. When sclerotia were germinated on media constantly readjusted to pH 1.3 and 6.7, respectively, mycelial development was more rapid at pH 1.3, but the rate of sclerotial formation was not affected. 3) Age of sclerotia. Sclerotia germinate most rapidly when newly formed, then more and more slowly until 10 days old, but thereafter gradually resume capability of prompter germination. When germination is most rapid it is also most uniform (at 31° C). Germination of 1-month-old sclerotia at 31° preceded that of 2-month-old ones by 24 hours. 4) Size and number of sclerotia. At 25° sclerotia were fewer but larger (1.5 mm) than at 31° (1.0 mm). At 37° sclerotia were very few and large (2.5 mm) but mostly abnormal. None were formed at 39°.

The effect of some growth-regulating substances on the development of a S. rolfsii isolate from apple was investigated by Lavee (8). Neither coumarin nor maleic hydrazide, at 1-100 ppm, had a pronounced effect on mycelial growth rates, but both had inhibitory effects on sclerotial germination; this was more marked with coumarin, which completely inhibited germination at 500 ppm. These substances reduced the number of sclerotia formed and in-

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[1] Publication of the National and University Institute of Agriculture, Rehovot, Israel, 1962 Series, No. 473-E. [2] Respectively, Senior Plant Pathologist at the National and University Institute of Agriculture, Rehovot and Head, Plant Protection Section, Extension Administration, Israel Ministry of Agriculture, Tel Aviv, Israel.

List of Hosts of Sclerotium rolfsii in Israel[a]

Allium cepa Bulbs affected chiefly at their neck; on onions sown in CB as late spring crops under irrigation. A. sativum Rare; in May-June in CB, JV. Amaranthus sp. 1 record, UG, Sept. Amaryllis sp. Dry bulb rot, 1 record, July. Antirrhinum majus Root, collar rot, Nov. Arachis hypogaea Widespread in CB, rare in UG. Moderately destructive on commonly grown Early Virginia variety. Roots, collars, gynophores, shells affected June-Sept. No field records of infections in first 2 months after sowing. Asparagus officinalis Root rot, 1 record, July. Aster sp. Recorded by Peleg (15). Beta vulgaris On autumn-sown beet rarely in Oct., mostly from Apr.; on spring-sown beet after not less than 10 weeks' growth. Common on light and heavy soils in CB and adjoining valleys, absent from chalky soil of JV. Calla sp. Dry tuber rot. 1 record, June. Cannabis sativa Collar rot. 1 record, June. Capsicum annuum Collar, root rot, common in CB, May-August. Transplants not affected in first 2 months in the field. CRUCIFERAE Cabbage, kohlrabi not affected. Cauliflower planted in Aug. sometimes affected in Sept. CUCURBITACEAE Cucumbers, vegetable marrow, watermelon not affected. Cucumis melo affected on fruit only, July-Sept. Cyamopsis tetragonoloba 1 record on seedlings, June. Cydonia vulgaris Peleg (15) recorded roots affected in nursery only. Cynara scolymus Collar rot, 1 record, Nov. Cynodon transvaalensis Roots occasionally affected, Sept. Dahlia variabilis Root, collar, corm affected, July-Aug. Daucus carota Severe in June-Sept. on unthinned carrots sown in late spring or summer in CB. Delphinium sp. Root, collar rot common in CB, Apr.-May. Dianthus caryophyllus Root, collar rot common in CB in Apr.-Nov., rare in Mar. Dichondra repens Collar rot, 1 record, Sept. Fragaria grandiflora Collar rot, 1 record in CB, July. Gladiolus sp. Corm rot, occasional in CB, June-Aug. Glycine max Collar rot, 1 record, Aug. Gossypium hirsutum, G. barbadense Only 3 records of collar, root rot in CB and UG, June. Helianthus annuus Rare. Affected plants continued growth without ill effects. H. tuberosus Collar, root rot, 1 record, Aug. Ipomoea batatas Tuber rot, 1 record, May. Iris spp. Dry bulb rot, common in CB in Mar.-Apr. Lycopersicum esculentum Collar, root rot widespread in CB, June-Sept. Rare in UG. Never recorded on seedlings or transplants less than 2 months old. Medicago sativa Root, collar rot, rare, Aug.-Sept. Narcissus sp. Bulb, root rot. Rare, late Mar.-June. Nicotiana tabacum Root rot. Rare, Aug.-Sept. Olea europaea Root rot. 3 records in June, Aug., Jan. Phaseolus vulgaris Root, collar rot common in CB, May-Oct. Prunus persica Root, collar rot. 1 record, Aug. Pyrus communis Root rot, 1 record, July. Pyrus malus Root, collar rot widespread in CB and adjoining valleys and in UG, from June onwards. Rheum undulatum Collar, leaf base rot. 1 record, July. Scabiosa sp. Collar, root rot, rare, June. Solanum melongena Collar, root rot widespread in CB in June-Sept. Never recorded on seedlings or transplants less than 2 months old. S. tuberosum Dry rot of stalk, collar, root, tubers. In CB and less in UG on spring crops from late Mar. to July, but not less than 6-7 weeks after sowing. On autumn crops seed pieces occasionally attacked, but plants otherwise free from infection for their first 4-5 weeks of growth. Trifolium alexandrinum Infrequent in CB, June. Tritonia sp. Collar, root rot, in CB, June-Sept. Valeriana officinalis 1 record, Apr. Vigna sinensis, 1 record, July. Viola odorata Rot of runners, 1 record, Oct. Vitis vinifera Root rot, 2 records in CB.

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[a] Abbreviations: CB = coastal belt, JV = Jordon Valley, UG = Upper Galilee.

creased their size. With coumarin this effect persisted for another generation, and sclerotia were darker than in the control. Inhibition was stronger on sclerotia of low viability. Alpha-naphthyl-acetic acid at concentrations as low as 2 ppm delayed sclerotial germination and reduced the number of sclerotia formed; at concentrations above 50 ppm the rate of mycelial growth was much lower, and the mycelium was unusually compact. This substance was the only one to hasten development of sclerotia. Triethanolamine did not affect the rate of mycelial growth, but 120 ppm and above increasingly inhibited germination of sclerotia. When these were of low viability, 1000 ppm sufficed to inhibit germination completely. This was a fungistatic effect since sclerotia subsequently transferred to substrates free from triethanolamine grew normally.

STRAINS IN ISRAEL Pathogenicity and cultural characteristics of a number of isolates from Israel hosts were studied by the senior author. Isolates from bean, cotton, carnation, chilli, Cannabis sativa, Dichondra repens, onion, potato, and apples, when grown on agar plates together with an isolate from tomato, failed to show the phenomenon of aversion described by Nakata (13) and Curzi (4). However, an isolate from violets showed distinct aversion when grown in culture with isolates from tomato, carnation, and apples. The isolate from violets in 4 years' successive culturing also developed fewer and larger sclerotia which formed later than in all other isolates.

In one cross-inoculation experiment, two isolates from chilli and one each from potato, tomato, and bean inoculated on 20 bean and 20 tomato plants all induced wilting in 50 to 75% of the beans and 45 to 70% of the tomatoes. In another experiment, isolates from tomato and violet were cross-inoculated on these two hosts. The isolate from violets killed 4 of the 16 violet plants inoculated, but none of the tomatoes. The tomato isolate killed all 16 tomato seedlings, but affected the violets only slightly on their lower leaves; these were attacked on their petioles and wilted, but the plant as a whole hardly suffered, and none of them were killed.

These observations indicate that at least two strains of S. rolfsii are present in Israel. One of these affects at least 10 hosts, and is widespread in the country, while the second appears restricted mainly to violets.

DISEASE DEVELOPMENT ON FIELD CROPS Conditions Necessary for Establishment of the Fungus: Successful establishment of S. rolfsii on host crops occurs where (a) a suitable strain is present, (b) inoculum has built up beyond a certain level, (c) the host is susceptible in the parts or tissues near the soil surface, and (d) environmental conditions favor pathogen development and do not unduly increase host resistance.

As far as strains are concerned, our survey indicates the prevalence of one or more strains with wide host ranges. Thus it is doubtful that failure of plants to become infected when sown on infested soil is due to the presence of an unsuitable strain. However, this factor cannot entirely be ruled out since cotton usually reported as susceptible (12, 21) abroad is rarely affected here.

Effect of Temperature and Moisture: In the rainless Israel summers development of the disease is limited, with rare exceptions, to crops irrigated in months with mean temperatures of 20° to 27° C, that is, from April to October. Even highly susceptible crops (carrots, sugarbeet), have only rarely been found affected where irrigation was concluded during months with lower temperatures. On late spring or summer crops that may be grown with or without irrigation (melon, tobacco, tomato, onion, cotton), S. rolfsii has never been recorded unless the crops were irrigated.

Humidity is clearly the limiting factor in the development of S. rolfsii under arid summer conditions, and this affects both the build-up of inoculum in the soil and subsequent invasion of hosts. In climates with summer rains it is not unusual for the fungus to attack its hosts in the seedling stage or at an early stage of growth. This occurs much more rarely in arid countries. In Israel, the pathogen is sometimes found on seedlings in densely sown nurseries of chilli or fodder beet, but in the field highly susceptible crops are mostly attacked at a later stage.

Under arid conditions the upper soil layer around young plants spaced suitably in rows dries up very rapidly after each irrigation. Even where plant debris is present near the collar of young plants, it is then unlikely to support active development of the fungus to the point where the build-up necessary for host invasion is reached.

However, as host plants develop more fully and increasingly shade the soil around their bases, the eco-climate near the soil surface becomes more and more favorable to the pathogen, as has also been pointed out by Magee (12). At the same time shedding of older leaves provides the plant debris so important for the build-up of inoculum (3). Here soil type and frequency of irrigation may also be of importance. In Israel S. rolfsii is common on both medium to heavy soils which dry slowly after each irrigation, and on light soils which dry rapidly but are irrigated at much shorter intervals. On the other hand, the disease is much less in evidence on loess soils where the upper layer dries rapidly as in light soils, but irrigation intervals approximate those on medium loams. Until now the only hosts affected on this type of soil are those that carry the pathogen on propagating parts or that are highly susceptible in the maturing stage (beets). However, most loess soils have been brought under irrigation only during the past 10 years, and may still be relatively free from infestation by S. rolfsii.

Host Susceptibility: Under suitable climatic and soil conditions, and on crops that do not carry the pathogen in their seed bulbs or tubers, attack of S. rolfsii in arid climates depends primarily on the susceptibility of host tissues near the soil surface when the plants have grown up to shade the soil sufficiently. Here hosts may be divided into three groups: (a) Hosts with tissues remaining markedly susceptible at all stages of maturation, especially sugarbeet and fodder beet, carrots, potatoes, iris, and carnations. Plants in this group often suffer severe attacks. All, except perhaps carrots, may be affected in April, although temperature in that month is not yet entirely favorable to the fungus. After irrigation is discontinued, S. rolfsii ceases to develop in that part of the sugarbeet root growing near soil level, while deeper parts continue to be affected. This was not observed in other plants, however. (b) Hosts with tissues near soil level gradually growing less susceptible. This group includes peanuts and beans, on which the damage caused is usually moderate and rarely severe, as well as chilli, tomato, and eggplant, on which damage is light to moderate. These crops are never affected before May, when temperatures become highly favorable to the pathogen. (c) Hosts with tissues near soil level rapidly becoming resistant; the outstanding example appears to be the cotton grown in Israel (mostly the varieties Akala 442, Akala 15/17, Koker and Pima). Sown in April, cotton plants even on badly infested soil have not been found affected in any month except June; they then reach the stage at which they provide sufficient shade while the stem may still be just susceptible enough to permit occasional penetration of the pathogen. On such a host the disease is without economic importance.

S. rolfsii has been observed in March only on those hosts known to carry the fungus in their seed material: rarely in potatoes, but more frequently in iris crops.

RESISTANCE AND SUSCEPTIBILITY IN APPLES Lavee and Samish (11) studied possible correlations between resistance to S. rolfsii and the physiology of various apple stocks. Examining the relative rate of infiltration of oxalic acid through the bark of stocks, they found acid uptake to be lowest in resistant and highest in the most susceptible stocks. When S. rolfsii was grown on media prepared from extracts of branches of various stocks, a brown color developed as the fungus spread. A correlation was found between the intensity of this color and the resistance of stocks to S. rolfsii. This method may thus serve as a bioassay for resistance in apple stocks. In further studies a chemical test consisting essentially in assessing the color of bark extract to which small amounts of $FeCl_3$ solution were added was developed to test for stock resistance.

Peleg, Barak and Frankel (17), in orchard trials carried out from 1949 to 1953, inoculated a total of 1700 apple stocks of 14 varieties. They found that 1) grafting the stocks with four varieties (Reine de Renette, Jonathan, Delicious, Grand Alexander) failed to produce any differences in susceptibility as compared with ungrafted stocks; 2) a positive correlation seems to exist between the vigor of tree development and resistance to S. rolfsii; 3) the stocks tested could be divided into five groups: (a) high resistance, Crab C; (b) medium resistance, East Malling stocks M IX, N XVI, M XV, and M XIII, and an Italian selection; (c) low resistance, East Malling M VII; (d) high susceptibility, East Malling M II, and the Italian selections T-I and T-I-b; (e) resistance variable according to the source from which the variety was received: Malus, and the indigenous varieties Hashabi and Succari. With Malus stocks, those received from France were consistently less susceptible than those grown in Israel.

Lavee and Samish (11), in further studies, inoculated 1-year-old apple stocks of seven varieties grown in tins. Ten weeks after inoculation statistically significant differences were obtained between the following three groups: (a) resistant: East Malling M XVI and M IX; (b) moderately susceptible: East Malling M XIII and M II, and the Italian selection T-I-b, and Hashabi; (c) highly susceptible: Succari. These differences were confirmed by bioassays and agreed well with orchard surveys of disease incidence on the various stocks.

CONTROL STUDIES Effect of Nitrogen Compounds: Peleg (16) studied the effects of gaseous ammonia and various ammonium salts (sulfate, carbonate, $NH_4H_2PO_4$ and $(NH_4)_2H_2PO_4$) on germination of sclerotia buried in pots filled with four soil types. Only the gaseous ammonia, and much less the ammonium carbonate, inhibited germination. The amount of gaseous ammonia required varied with the type of soil; application of the gas at the rate of 150 ppm of soil weight killed 90% of the sclerotia on sandy soil, whereas six times as much was required on heavy soil.

Lahover and Avizohar-Hershenzon (5) investigated effects of aqueous ammonia on germination of sclerotia buried 15 cm deep. Treatments were applied to three soil types contained in barrels, at rates corresponding to 460 and 1850 kg N per hectare, respectively. Even the lower rate of application reduced sclerotial germination by about half but the higher rate gave significantly better results, and practically stopped all germination at the lowest soil level tested (10 to 15 cm). Application by flooding was superior to "furrow" irrigation at the lower, but not at the higher, rate of application. Best results were obtained by split treatments at rates of 925 + 925 kg N per hectare, applied by furrows, with the top soil buried by cultivation in the interval between treatments.

However, in contrast to the above findings, Lavee (10) was unable to detect a beneficial effect of N application in curtailing development of this disease on apple. M II applestocks supplied with organic manure or ammonium sulfate (at 600 kg per hectare) did not differ significantly in disease incidence from stocks given both manure and fertilizer or control plots in which no nutrients were applied.

Soil Amendments and Cultural Studies: Introduction of cover crops and non-tillage practices into Israeli apple orchards resulted in a dramatic decrease in losses due to S. rolfsii; the disease was practically eliminated from all orchards brought under this management, even from those in which high percentages of trees had previously been removed because of S. rolfsii. In one typical case, cited by Lavee (9), an apple orchard planted to trees on Doucin stock in 1947 on heavy soil was severely affected by the disease. Five years after planting a cover of alfalfa was sown. In that year 6.8% of the trees were killed by the fungus, and another 5.1% died the following year. But, during the following 3 years the percentages of trees killed fell to 3.4, 0.4 and 0, respectively, while in orchards without cover crops trees continued to succumb at an undiminished rate.

After some 12 to 15 years' experience in non-tillage management of orchards, the following practice is now generally accepted in Israel as insuring freedom from S. rolfsii attack (14). During the first 1 to 2 years after planting, when trees are still irrigated by applying the water to basins prepared around each tree, a straw mulch is applied to that basin only. Thereafter, when overhead irrigation of the whole orchard area begins, crops of perennial rye grass are sown. This has largely superseded the sowing of alfalfa, because the rye grass has shallow roots that do not interfere with orchard growth and because it is less prone to the attack of pests dangerous to apples, for example, Prodenia litura. The rye grass ceases growth in summer, but renews its growth in autumn by self-seeding. The mulch of dry grass persisting in summer, together with some weed cover, adequately prevents S. rolfsii development. The question then arose, whether cover crops and non-tillage produce their beneficial effect by 1) stimulating microflora antagonistic to S. rolfsii, 2) reducing mechanical dispersion of sclerotia in the soil, 3) changing soil temperature, or 4) increasing host resistance.

In an investigation by S. Lavee (9), 1-year-old M II apple stocks were planted in sandy loam in containers. A mulch of fine wood shavings was applied to some of the stocks. When this was found to reduce temperature in the upper 5 cm of soil by up to 11° C (in August), a heating cable was introduced in some of the mulched containers 5 cm under the soil surface, and temperatures there were raised to the level of the soil around the unmulched stocks. Plants were inoculated 10 days after mulching with mature sclerotia.

Results indicated that mulching markedly increased vigor of root and shoot growth and greatly reduced mortality caused by S. rolfsii. Although most of the stocks under mulch had their roots superficially attacked, the fungus failed to penetrate them beyond the outermost layer of bark. This is all the more remarkable since mycelial development under the mulch was profuse. Heating the soil cancelled the beneficial effect of the mulching, caused stocks to be attacked to the same extent as unmulched stocks, and even advanced the rate at which plants were killed. Bioassays of the resistance of stocks gave significantly higher values for stocks on mulched and unheated soil than for those on mulched and heated or on unmulched soil. The author concluded that mulching checked S. rolfsii development on apple stocks primarily by reducing soil temperatures which rendered the stocks more vigorous and thus reduced their susceptibility to attack by this pathogen.

Corroboration of this view is also found in the fact that young apple stocks are rarely attacked earlier than June. As shading of the soil cannot be important with young trees spaced widely apart, Lavee's conclusion that apple roots are attacked only when rendered susceptible by high temperatures may explain why apple stocks are affected considerably later in the season than any of the susceptible field crops.

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EFFECTS OF STING AND STUBBY-ROOT NEMATODES ON ST. AUGUSTINE GRASS[1]

H. L. Rhoades[2]

Abstract

In a greenhouse experiment, inoculations of 500 and 1000 Belonolaimus longicaudatus Rau, 1958 in 6-inch pots of Leon fine sand reduced growth significantly and caused severe chlorosis of common St. Augustine grass (Stenotaphrum secundatum) during an 8-month period. Root weight was reduced 62 and 69%, respectively, for the two infestation levels during the same period. In another test, an inoculation of 1000 Trichodorus christiei Allen, 1957 reduced growth 25% and root weight by 49% during 5 1/2 months.

INTRODUCTION

Plant parasitic nematodes have been recognized as severe pests of turf grasses in numerous areas of the United States during the last few years. In Rhode Island, Tarjan and Ferguson (9) found Panagrolaimus and Eucephalobus spp. associated with a yellow turf condition of bentgrass. Troll and Tarjan (10), in surveys of golf course greens, stated that Tylenchorhynchus claytoni and Rotylenchus erythrinae were the most common plant nematodes associated with decline of turf in Rhode Island and found several previously unreported nematode parasites of grasses. Parris (7) reported species of Tylenchorhynchus, Rotylenchus, Trichodorus, Criconemoides, Xiphinema, Pratylenchus, Hoplolaimus, and Paratylenchus attacking lawn grasses in Mississippi. Jenkins, et al. (5) reported a number of parasitic and nonparasitic nematodes associated with grasses in Maryland. In a survey of Georgia turf nurseries, Good, et al. (4) found species of Trichodorus, Xiphinema, Helicotylenchus, Pratylenchus, Criconemoides, Tylenchorhynchus, Belonolaimus, Hoplolaimus, and Tylenchus occurring frequently around the roots of grasses. In addition, species of Aphelenchus, Aphelenchoides, Paraphelenchus, Paratylenchus, and Ditylenchus occurred occasionally. In Florida, Kelsheimer and Overmann (6) reported B. gracilis, Trichodorus sp., Criconemoides spp., Hoplolaimus coronatus, R. similis, and D. dipsaci associated with root injury and turf decline of St. Augustine grass. Christie, Good, and Nutter (1) and Good, Christie, and Nutter (3) also found species of Rotylenchus, Xiphinema, Dolichodorus, Pratylenchus, and Hemicycliophora attacking turf grasses.

Although plant parasitic nematodes have been frequently associated with turf injury, very little experimental evidence has been published on damage caused by specific nematode species parasitizing specific grasses. Perry (8), in greenhouse inoculation experiments and histopathological studies, found that decline and root injury of Kentucky blue grass in Wisconsin was caused by Helicotylenchus spp.

During 1960-61, pathogenicity experiments were conducted in the greenhouse at Sanford, Florida in which St. Augustine grass (Stenotaphrum secundatum), one of the most popular lawn grasses in Florida, was inoculated with two levels of Belonolaimus longicaudatus Rau, 1959 and one level of Trichodorus christiei Allen, 1957. These two nematodes are frequently found at high population levels and associated with injury to St. Augustine grass in the Sanford area.

MATERIALS AND METHODS

Experiment 1: To obtain cultures free from plant parasitic nematodes, stolons of common St. Augustine grass growing out over a concrete driveway were cut and planted two per pot in 6-inch clay pots of autoclaved Leon fine sand in the greenhouse on September 23, 1960. They were planted with two nodes below the surface of the soil, then kept well watered until roots had formed and growth resumed.

On October 18, the grass was cut back to 2 inches above the pot rim and a randomized block test with five replicates established. Treatments were: 1) no nematodes, 2) 500 B. longicaudatus per pot, and 3) 1000 B. longicaudatus per pot. The nematodes used in the experiment were taken from a population reared on snap beans in the greenhouse and were obtained by processing the soil by the modified Baermann technique of Christie and Perry (2). Decanting and a second passage of the nematodes through a pad of 4-ply facial tissues freed them essentially from all residues from the old culture. Twenty-five ml of supernatant liquid from above the settled nematodes were added to each of the check pots after being passed through filter paper to insure freedom from nematodes or their eggs. Presumably, this supernatant carried bacteria of kinds that also were associated with the nematodes.

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The experiment continued until June 11, 1961, during which time the pots were fertilized three times (December 1, March 9, and May 3) with 1 gram per pot of a 5-5-7 fertilizer containing traces of secondary elements. The grass was clipped back to 2 inches high six times (December 9, March 9, April 12, May 3, May 24, and June 11) and the clippings weighed each time. After the last clipping was made, the soil ball was knocked from each pot. Soil samples were taken with a sampling tube from both sides and the bottom of the ball for nematode counts; the roots were washed free of sand, blotted, and weighed.

Experiment 2: This experiment was handled in the same manner as Experiment 1, except that only one infestation level (1000 *T. christiei* per pot) was used. These nematodes had been cultured on sweet corn in the greenhouse. Five pots were infested on January 10, 1961 and five had only supernatant liquid from above the settled nematodes added. The experiment continued through June 11, 1961, during which time the pots were fertilized twice (March 9 and May 3) and clipped five times (March 9, April 12, May 3, May 24, and June 11).

RESULTS AND DISCUSSION

Experiment 1: From October 18, 1960 to March 9, 1961 no symptoms were evident in the grass in pots infested with *B. longicaudatus*, and weights of the two clippings during this interval were nearly the same for all treatments. As nematode populations increased, however, and as growth of the grass accelerated due to higher temperatures during the spring months, grass in infested pots became quite chlorotic and weights of the last four clippings were significantly less than the checks (Table 1, Fig. 1). Root growth was greatly reduced (Table 1) and there were severe abnormalities due largely to devitalized root tips and injury inflicted along the sides of the roots (Fig. 2). Symptoms included stubby root, necrosis, discoloration, and lesions.

Nematode counts at the close of the experiment showed that the population had increased to 4048 for pots originally infested with 500 nematodes and to 4114 for those infested with 1000. This indicates that the grass may have been unable to support a higher population under the conditions of this test.

Experiment 2: Reduced growth was the only aboveground symptom exhibited by the grass infested with *T. christiei*. Clipping weights showed that growth had been reduced 25% by the nematodes; root weight was reduced by 49% (Table 2). The major effects on the root system were reductions in size with fewer and shorter rootlets and stubby tips on the rootlets (Fig. 3). No discoloration or necrotic lesions were evident. The nematode population increased from 1000 to 11,778 during the experiment.

Results of these experiments indicate that *B. longicaudatus* and *T. christiei* are serious pathogens and important economic pests of St. Augustine grass in Florida.

Table 1. Effect of *Belonolaimus longicaudatus* on the growth of common St. Augustine grass.

Inoculation level	Total clipping weight Oct. 18-Mar. 9[a] (in grams)	Total clipping weight Mar. 9-June 11 (in grams)	Root weight (in grams)
No nematodes	36.6	58.3	104.4
500 nematodes	39.4	45.2	39.5
1000 nematodes	34.0	42.0	32.1
LSD .05		7.3	12.1
.01		10.6	18.4

[a] Total weight clipped from individual 6-inch pots (average of five replicates).

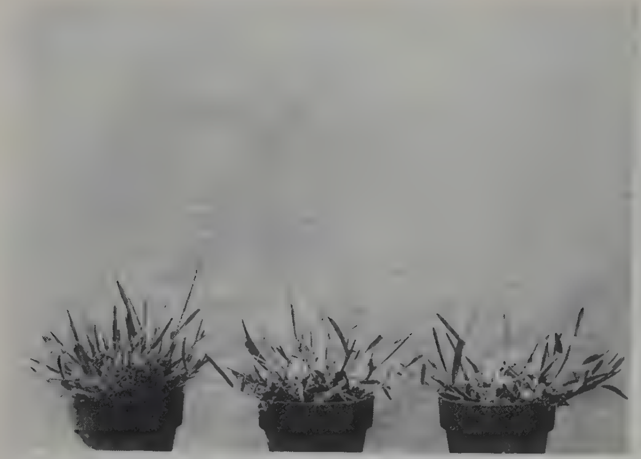

FIGURE 1. The effect of B. longicaudatus on the growth of St. Augustine grass. Left -- no nematodes. Center -- 500 nematode infestation level. Right -- 1000 nematode infestation level.

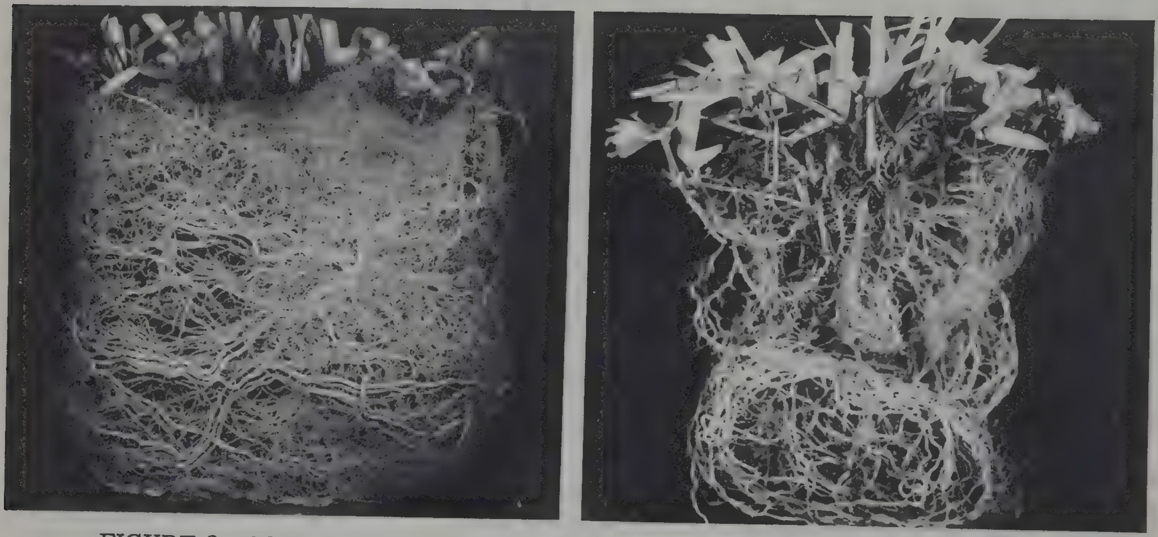

FIGURE 2. The effect of B. longicaudatus on root growth of St. Augustine grass. Left -- no nematodes. Right -- 1000 nematode infestation level.

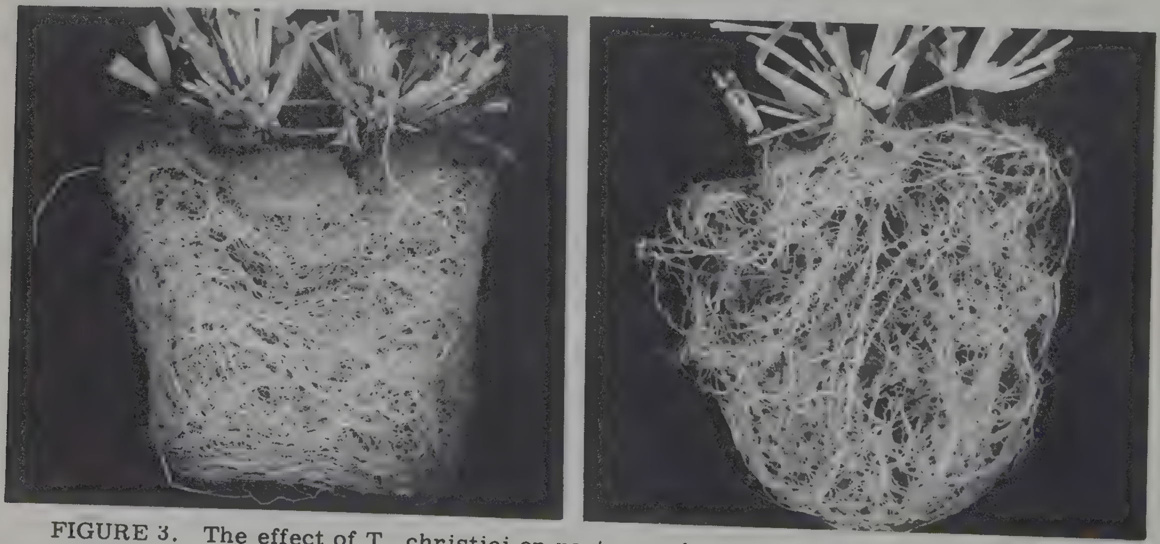

FIGURE 3. The effect of T. christiei on root growth of St. Augustine grass. Left -- no nematodes. Right -- 1000 nematode infestation level.

Table 2. Effect of *Trichodorus christiei* on the growth of common St. Augustine grass.

Inoculation level	Total clipping weight Jan. 10-June 11[a] (in grams)	Root weight (in grams)
No nematodes	43.4	66.7
1000 nematodes	32.6	34.1

[a] Total weight obtained from individual 6-inch pots (average of five replicates).

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EFFECTS OF BIG VEIN ON THE IRRIGATED HEAD LETTUCE CROP[1]

R. B. Marlatt and R. T. McKittrick[2]

Summary

Furrow-irrigated head lettuce was grown in a field with a history of big vein and plants showing symptoms were compared with symptomless plants. At the first harvest, symptomless plants yielded five times as many firm heads. After the last harvest total number of marketable heads were the same. Size of nearly mature diseased plants was reduced as shown by smaller diameters, fewer leaves and lighter weights 10 days before harvest. Harvested, diseased heads were also lighter. Plants with big vein yielded as much seed as symptomless plants and the germination rate and percentage were about the same. Roots of diseased and symptomless plants were equally infected by Olpidium sp. at harvest time.

INTRODUCTION

Big vein is a virus disease reportedly transmitted by a fungus, Olpidium brassicae (Wor.) Dang. (1, 9). It has been observed in irrigated lettuce since 1917 (8), but its effect upon field-grown plants has not been thoroughly studied. Although no measurements were previously reported, the disease has been blamed for diminished head size and quality of field-grown lettuce (4, 5, 6, 10, 12). The longer the period during which plants were diseased in a greenhouse, the less they eventually weighed (8). Delayed head maturity is reportedly another result of field infection by big vein (6, 12). No studies of the effect of big vein upon seed production or germination have been reported.

METHODS AND MATERIALS

Lettuce was grown on the farm of the University of Arizona Mesa Branch Station and commercial cultural practices were followed. In this warm desert area one or two crops of lettuce are grown during the season from August to May in two rows per raised bed; beds are irrigated by furrows running between them. Great Lakes 66 variety was used in the 1959-60 season and Premier Great Lakes in 1960-61, with approximately 2 acres of lettuce grown for each annual experiment. As plants began to head they were identified with stakes as symptomless or diseased. A total of 430 plants were studied in 1959-60 and 699 in 1960-61, approximately half were diseased. Soil temperatures 6 inches under the seed row remained below 60° F from December to May and were satisfactory for Olpidium infection.

RESULTS

Number of Heads Harvested: Firm, mature heads were harvested and the total diseased or symptomless recorded for each of several harvest dates.

At the first harvest, symptomless plants yielded approximately five times as many heads as did diseased plants. Group comparisons showed this to be statistically significant. Totals of all harvests did not differ significantly because most diseased plants later formed firm heads.

Maturation Rate: The number of heads obtained at each harvest was multiplied by the number of days required for them to mature and these products were subjected to group comparison. Diseased plants required approximately 136 days to mature heads whereas symptomless plants required 131 days. Because of high variability within each group, this difference was not statistically significant. Since many more symptomless heads were obtained at the first harvest, maturation was apparently delayed by the disease.

Plant Size: During the first year, 10 days before the first harvest, plant size was estimated by measuring plant diameters, by counting the leaves on the plants and by obtaining top weights. Averages for diseased lettuce were 9.5 inches diameter, 33.6 leaves, and 0.40 pound; symptomless averages were 10.9 inches, 36 leaves, and 0.53 pound. Stunting by the disease as shown by each of these measures of plant size was statistically significant by group comparison.

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[1] Contribution from the Arizona Agricultural Experiment Station, Journal Paper No. 723.

[2] Associate Plant Pathologist and Assistant in Research.

Weights of mature heads from all harvests were recorded the second year. Symptomless heads averaged 0.11 pound heavier than diseased, statistically a very significant difference.

Seed Yield: Seed produced by 701 plants was harvested, cleaned and the total production of each plant weighed separately; approximately half of the plants had big vein. Yield from symptomless plants averaged 4.46 g and diseased plant yield averaged 4.09; however, this difference was not statistically significant. Apparently big vein did not diminish seed production.

Seed Germination Rate and Percentage Germination: Testing procedures were devised by reference to the U. S. Department of Agriculture Handbook No. 30 (11) and followed extensive preliminary trials. Seeds were chilled for 3 days at 55° F and then germinated in Petri dishes on filter papers moistened with a 0.2% aqueous solution of potassium nitrate. Each dish containing 25 seeds was stored in a dark constant-temperature box in 100% relative humidity at 68°. Seeds were exposed for 30 minutes daily to 120 fc of white fluorescent light (Sylvania 40 watt daylight lamps). Daily records of germination were kept for 7 days. A final count of normal seedlings was made on the eleventh day to provide the germination percentage. The average number of days required to show first signs of germination, that is, rate of germination, was recorded for each dish. Over 7000 seeds were tested, half from diseased and half from symptomless plants. Seed from symptomless plants began to germinate after 1.18 days and from diseased plants after 1.15 days; percentage germination averaged 91.6 and 92.6, respectively. Group comparisons detected no statistically significant differences in either rate or percentage germination. Big vein apparently did not influence germination of seed.

Root Infection: Big vein reportedly is transmitted by a fungus. Symptomless plants have been found with infected roots (2, 3) and soil from under them has been used for transmission of the disease (10). The extent of root infection in symptomless and diseased lettuce plants was compared when heads were mature.

Estimation of the extent of root infection was similar to the method used for estimating infection by Plasmodiophora brassicae Wor. (7). Three rootlets less than 5 mm long from each of 150 diseased and 150 symptomless plants were examined microscopically for Olpidium. Plants were rated 0, 1, 2, or 3, depending on the number of rootlets containing sporangia or resting spores. An average of 1.01 of every three rootlets on symptomless plants were infected and 1.21 on diseased plants. Group comparisons were made of the ratings and of numbers of plants infected. No statistically significant differences were found, indicating that most plants were infected by the fungus at harvest time regardless of the presence or absence of symptoms of the virus disease.

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EVALUATION OF CHEMICALS FOR SYSTEMIC ACTION IN CITRUS SEEDLINGS INFECTED WITH RADOPHOLUS SIMILIS[1]

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Abstract

A number of chemicals were evaluated for systemic therapeutic action. Twin rooted citrus seedlings, to which a chemical was applied to one-half of the root system and nematodes to the other half of the root system, provided a means of assessing host and pathogen response. There was no elimination of R. similis in any of the treatments that promoted good host development. Host vigor appears to be a good criterion for assessing the therapeutic merits of a chemical. Of the growth regulants tested, 2 methyl-1-naphthylene acetic acid and N-o-chlorophenyl phthalamic acid were the best. Of the non-growth regulants, $CaCl_2$, tributyltin o-phenylphenate and O,O-diethyl-O-2-pyrazinyl phosphorothioate were the most effective. All five chemicals stimulated development of both root systems.

INTRODUCTION

The pull and treat program is presently the only method of controlling or curtailing spreading decline of citrus caused by the burrowing nematode, Radopholus similis (Cobb) Thorne (11). To find alternatives to this program, various in-place tree treatment studies have been undertaken to (a) eradicate R. similis, (b) prevent its feeding on the roots of the host, or (c) alter the life processes of the pathogen to the benefit of the affected tree. In these in-place treatment programs, three basic techniques are employed: (a) soil treatment of a limited area around each tree (8, 12), (b) sprinkler irrigation of several acres or more with either 1,2-dibromo-3-chloropropane (DBCP) or O,O-diethyl O-2-pyrazinyl phosphorothioate (Cynem) (13, 14) and (c) mechanical injection of the toxicant into the trunk so as to facilitate systemic action (9, 15). No material was found in any of these systemic trials that would control or repel R. similis from infected citrus trees. Although the application of toxicants by the sprinkler irrigation method gave better nematode control and was less phytotoxic than the "limited area" treatments, the irrigation trials gave only "partial control," that is, no nematodes were found on any feeder root samples obtained at a depth of 1 to 1 1/2 m during the first year following irrigation with DBCP and for approximately 6 months following irrigation with Cynem. This suggests that both toxicants had possibly penetrated the soil and feeder roots in sufficient concentration to control R. similis to a depth of at least 1 1/2 m. Nematodes and/or eggs protected by larger roots or in the soil below 1 1/2 m were unaffected and presumably served as reservoirs for infections.

The deep sands of the central Florida ridge section is where spreading decline is most prevalent. Citrus tree roots penetrate these soils to a depth of 5 to 7 m and it is believed that citrus roots might find a suitable medium where the soil profile is 10 m deep. The burrowing nematode has been recovered from citrus roots 4 m deep. Temperature data from soil depths to 3.3 m (unpublished, E. P. DuCharme) lend credence to the belief that R. similis can live in 5 m or deeper soil areas where the temperature remains approximately 21° to 27° C.

Because of the soil depth and the difficulty of penetrating it with chemicals, the root system appeared to be an acceptable means of transporting chemicals. Soil application of chemicals was also believed to provide more opportunity for success than foliage treatment with its inherent difficulties of absorption and translocation.

Since it is difficult to establish an experimental system for evaluating soil applications in the greenhouse so as to approximate the problem encountered in the field, it was necessary to find a suitable substitute. Citrus seedlings with twin roots (3, 10) make it possible to use the root system as a transporting medium, and hence to evaluate chemicals for both host and pathogen response without direct chemical contact with the pathogen. Thus, the two root systems on a single stem provide a continuous anatomical and physiological connection, although each root system is in its own soil mass. This is somewhat comparable to treating the soil under a citrus tree where R. similis is some distance from the area of chemical application.

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During the past 3 years, 56 chemicals have been tested on twin-root citrus seedlings for therapeutic action against R. similis in the greenhouse and in soil temperature tank experiments. This paper summarizes the host-pathogen response in these tests and presents the possibility that the disease may be mitigated even though the pathogen is not eliminated.

PROCEDURE

Screening Program: The experiments were conducted in the greenhouse during the late fall and winter months to avoid the deleterious effects of sustained high temperatures (above 33° C) on R. similis. To prepare the twin-root plants, the tap root of grapefruit seedlings (Citrus paradisi var. Duncan) 7 to 9 mm/d was split lengthwise from the tip to the base of the stem. Each half-root system was then planted in a 46-oz paired-can of steamed Lakeland soil. The cans were secured by crimping the adjacent tops and by tying the bases with wire. Seedlings were allowed to callus and become established for 3 to 4 months before use. Over half of the chemicals selected for the screening tests are growth regulators or related materials since several growth regulants have exhibited systemic therapeutic action against diverse fungus pathogens (1, 2, 4, 5). The organic chemicals, unless supplied as formulated materials, were prepared in 2 to 5 ml of 95% ethanol with one drop of wetting agent (Tween-20) and made to a final volume of 100 ml with water. This volume was applied to the soil of one of the twin roots of each plant. Treatments were applied three times, at 1-month intervals. The portion of the root system not receiving chemical treatment was inoculated with 60 to 75 mature female burrowing nematodes 30 days after the first chemical application. Each half of the root system was supplied with 50 ml of liquid fertilizer every month. The following proportions were used in 4000 ml of water: Nutri-leaf[3], 17 g; $MgSO_4$, 3 g. Each treatment was replicated three times. Plants were harvested 4 months after the last chemical application. Data were taken on plant height, weight of each root system, phytotoxicity, and number of R. similis (16).

Temperature Tank Studies: Temperature tank tests with twin-root plants were made, for the most part, with those chemicals that showed good host response in the greenhouse screening trials. Ambient root temperature was maintained at 26° C and all treatments were replicated six times. The same procedures for applying chemicals, fertilizers and inoculum as used in the screening trials were employed in the temperature tank studies. Data on top weight, weight of each root system, phytotoxicity, and number of R. similis were taken 5 months after the last treatment.

RESULTS

Screening Program: In no case did any treatment eliminate R. similis. Because of the inherent variability of nematode populations, it was not possible to attach any significance to the number of R. similis recovered.

Of the growth regulants, the phthalamic acid derivatives, indole derivatives, and 2-methyl-1-naphthalene acetic acid appeared to be the best (Table 1). The root systems of plants receiving these treatments were well developed and the root side inoculated with R. similis had little or no characteristic discoloration. These plants also had good top growth and showed no symptoms of spreading decline. Calcium chloride at 1000 lb/A and tributyltin o-phenylphenate at 20 lb/A appeared to be the best of the non-growth regulants (Table 1). Plants in these treatments showed good root color and good root development on both the chemical and R. similis inoculated sides. Development of tops was better in the calcium treatment than in the tributyltin treatment.

Temperature Tank Tests: There was no elimination of R. similis in any of the treatments that promoted good plant development. Best host responses were obtained with N-o-chlorophenyl phthalamic acid 20 lb /A, N-p-nitrophenyl phthalamic acid 20 lb/A, tributyltin o-phenylphenate 20 lb/A, 2 methyl-1-naphthylene acetic acid 10 lb/A, and O,O-diethyl O-2-pyrazinyl phosphorothioate (Cynem) 12 lb/A (Table 2). These five chemicals stimulated root development on both the R. similis inoculated and chemically treated sides. The naphthylene derivative and Cynem were superior in over-all host development. Only a few necrotic areas were discernible on the R. similis inoculated root side of plants treated with either of the latter two materials. The increase in root development following Cynem treatments was also observed in field trials (14). Terminal growth and leaf development comparable to the healthy controls were obtained with N-o-chlorophenylphthalamic acid and tributyltin o-phenylphenate.

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[3] Miller Chemical Company, Baltimore, Maryland.

Table 1. Response of twin-root grapefruit seedlings to chemicals applied to one root system and *R. similis* inoculated on the other root system. Greenhouse experiments.

Treatment	Rate lb/A[a]	Plant rating[b]	*R. similis* per gram root[c]	Treatment	Rate lb/A[a]	Plant rating[b]	*R. similis* per gram root[c]
N-(m) nitrophenyl phthalamic acid	100	5	6.6	Tetramethylguanidine	50	0	1.0
do.	20	5	4.0	do.	20	0	2.3
N-(p) nitrophenyl phthalamic acid	100	5	2.4	Pyridinethione disulfite	50	0	0.5
do.	20	5	2.0	do.	20	0	20.6
N-(o) chlorophenyl phthalamic acid	100	2,3	1.3	Sodium 2-pyridinethiol-1-oxide	50	0	38.6
do.	20	5	2.6	do.	20	0	15.3
N-(p) chlorophenyl phthalamic acid	100	5	1.8	Vancomycin-HCl	50	1	7.2
do.	20	5	8.1	do.	20	1	5.9
N-(2,3) dichlorophenyl phthalamic acid	100	5	1.6	Actidione-HCl	50	0	5.3
do.	20	5	1.8	do.	20	0	4.3
N-(3,4) dichlorophenyl phthalamic acid	100	5	3.8	2-chloro-4-fluorophenoxy acetic acid	50	0	---
do.	20	5	2.2	do.	20	0	14.0
N-(2,3,5) trichlorophenyl phthalamic acid	100	5	1.4	2-chloro-4-fluorophenoxy acetic acid (N-butyl ester)	50	0	---
do.	20	5	1.0	do.	20	0	4.3
N-(3,4,5) trichlorophenyl phthalamic acid	100	1	5.6	2-chloro-4-fluorophenoxy acetic acid (2-chloro ethyl ester)	50	0,3	4.5
do.	20	5	1.0	do.	20	0,3	13.5
N-1-naphthyl phthalamic acid	50	1	6.6	2-chloro-4-fluorophenoxy acetic acid (dimethylamine salt)	50	0	4.6
do.	20	1	6.3	do.	20	0	5.5
Pyroguanazole[d]	100	2,4	5.0	4-(2-chloro-4-fluorophenoxy butyric acid) (N-butyl ester)	50	0	23.6
do.	20	2,4	5.1	do.	20	0	6.8
3,5 dichloro-1,2,4-triazole	100	2,3	3.9	4-(2-chloro-4-fluorophenoxy butyric acid) (2-chloro ethyl ester)	50	0	3.2
do.	20	2,3	3.3	do.	20	0	2.4
Guanazole[e]	100	1	6.4	4-(2-chloro-4-fluorophenoxy butyric acid) (dimethylamine salt)	50	0	12.2
do.	20	2	3.5	do.	20	0	9.7
Calcium chloride	5000	0	---	2-chloro-4-fluorophenoxy ethanol (sodium sulfate)	50	0	9.3
do.	2500	1	4.1	do.	20	0	28.6
do.	1000	5	3.8	2-chloro-4-fluorophenoxy ethanol (trichloro acetic acid ester)	50	0,3	19.0
3,5,5 trimethyl-1-hexanol	50	0	1.5	do.	20	0,3	21.0
do.	20	1	4.4	2-chloro-4-fluorophenoxy ethanol (benzoic ester)	50	0	10.5
Hesperidine haphthylamine	50	1	12.0	do.	20	0	4.6
do.	20	1	4.4	4-(2-chloro-4-fluorophenoxy) butanol	50	0	27.2
Hesperidine m-toluidine	50	1	4.4	do.	20	0	28.4
do.	20	1	8.5	4-(2-chloro-4-fluorophenoxy) butanol (sodium sulfate)	50	0	2.7
Hesperidine 2,5 dichloroanaline	50	1	8.3	do.	20	0	16.6
do.	20	1	4.0	4-(2-chloro-4-fluorophenoxy) butanol (trichloro acetic acid ester)	50	0,3	32.2
Sodium 2,3,6-trichlorophenyl acetic acid	50	0	5.6	do.	20	0,3	1.8
do.	20	0	1.5	4-(2-chloro-4-fluorophenoxy) butanol (benzoic ester)	50	0,3	21.0
P-tert butyl phenyl	50	1	3.0	do.	20	0	22.5
do.	20	1	3.0	L-methionine octadecyl sulfate	50	1	13.0
Triphenyl tin acetate	50	1	4.0	do.	20	1	21.0
do.	20	1	1.0	FOAH[f]	50	1	44.7
Tributyltin	50	1	15.5	do.	20	1	25.7
do.	20	1	5.0	Naphthalene α propionic acid	50	2,3	18.6
Tributyltin ortho phenylphenate	50	1	2.5	do.	20	1	37.7
do.	20	5	2.0	Naphthalene acetic acid	50	2,3	23.7
Ammonium bromide	300	1	2.2	do.	20	1	9.7
do.	100	1	12.2	2-methyl-1-naphthalene acetic acid	50	1	19.4
1,2-dibromo-3-chloropropane	8 gal.	0	1.6	do.	20	5	22.9
do.	4 gal.	1	1.4	Indole acetic acid	50	5	4.7
2,3-dichlorophenoxy pelargonic acid	50	0	14.0	do.	20	1	18.9
do.	20	0	8.9	Indole butyric acid	50	5	14.5
Sodium hexylene glycol monoborate	50	0	7.0	do.	20	2,4	53.1
do.	20	0	1.4	2,4-dichlorophenoxy α propionic acid	50	0	21.0
Dimethyl 1,2-dibromo-2,2-dichloroethyl phosphate	50	0	2.7	do,	20	0	3.7
do.	20	0	13.1	Controls	--	-	17.5

[a] Rate/A for each of the three treatments applied.

[b] Rating - 0. Phytotoxic. 1. No improvement as compared with non-treated decline seedlings. 2. Improved top growth. 3. Improved root growth on inoculated side. 4. Improved root growth on side of chemical treatment. 5. Improved growth of entire plant.

[c] Data on nematodes calculated from weight of roots on inoculated side.

[d] Pyroguanazole = 3,4 dichloro-1,2,4-triazole.

[e] Guanazole = 2,3 dichloro-1,2,4-triazole.

[f] FOAH = an emulsified fish oil.

Table 2. Response of twin-root grapefruit seedlings to chemicals applied to one root system and R. similis inoculated on the other root system. Temperature tank experiment.

Treatment	Rate lb/acre[a]	Top weight (in grams)	Root weight (in grams) inoc. side	Root weight (in grams) chem. side	Root weight (in grams) Total	R. similis per gram root[b]
N-m-nitrophenol phthalamic acid	50	72.7	29.5	33.0	62.5	8.6
do.	20	66.0	30.6	32.5	63.1	8.3
N-m-tolyl phthalamic acid	50	73.6	31.9	30.2	62.1	8.9
do.	20	66.1	24.0	28.5	52.5	5.3
N-o-chlorophenyl phthalamic acid	50	81.5	33.8	33.4	67.2	4.8
do.	20	91.1	33.9	38.5	72.4	3.8
N-p- nitrophenyl phthalamic acid	50	73.9	25.9	32.2	58.1	7.2
do.	20	81.0	32.2	37.2	69.4	7.1
Tributyltin o-phenylphenate	20	90.0	30.6	35.2	65.8	3.7
2 methyl-1-naphthylene acetic acid	10	80.0	36.0	44.3	80.3	8.7
2-chloro-4-fluorophenoxy acetic acid	2	35.0	24.6	21.8	46.4	8.0
1,2-dibromo-3-chloropropane[c]	103	78.2	42.2	31.1	73.3	1.0
O,O-diethyl O-2-pyrazinyl phosphorothioate[d]	12	80.0	43.0	46.4	89.4	5.0
Control (one root system inoculated)	--	76.8	23.6	34.5	58.1	4.2
Control (both root systems inoculated)	--	81.4	29.9	32.6	62.5	4.6
Control (not inoculated)	--	90.6	35.3	34.4	69.7	---

[a] Rate/A for each of the three treatments applied.

[b] Calculated for roots on inoculated side.

[c] Nemagon.

[d] Cynem.

DISCUSSION AND CONCLUSIONS

The use of twin-root plants to evaluate the effect of chemicals on the host and pathogen appears to have merit in the present study of spreading decline and may possibly have some use in the study of other root diseases. In these experiments certain growth regulants, specifically the phthalamics, indoles, and naphthalene derivatives, stimulated the host in spite of no reduction of the population of R. similis. It has been found that certain fungi, notably Fusarium (7), as well as Rhizoctonia and Sclerotium (unpublished, E. P. DuCharme) have been consistently associated with R. similis and that the severity of decline is aggravated by the nematode-fungus association. Various workers have shown that growth regulating materials can be used as therapeutants against several fungi, including Fusarium (1, 2, 4, 5, 6). The mechanism for host stimulation by the growth regulators used in our tests is not known at this time. These chemicals may have ameliorated the effects of R. similis and/or the associated fungi by modifying the nature of the host's pectins (2, 6), or perhaps by neutralizing certain metabolites toxic to citrus. It is also possible that the host was stimulated in such a manner that new roots were developed at a faster rate than the rate of root destruction by the nematode.

Of the non-growth regulating chemicals used in the twin-root tests, calcium and tributyltin o-phenylphenate appeared to promote the best host vigor. The effect observed with calcium is especially interesting in light of recent work on Fusarium wilt of tomato in which calcium was shown to increase host resistance in a manner similar to that suggested for certain growth regulators (2, 6). It is much too early to speculate whether calcium and the growth regulants have a similar mode of action against the R. similis-fungus complex on citrus. Since healthy and diseased plants do not always show comparable response to the same chemical treatment, additional tests on rates and sources of calcium on both healthy and decline citrus are currently under investigation.

The question of whether it is possible to reduce the ravages of R. similis without eliminating it is of considerable importance. If a treatment can be used that will allow the host to function normally or nearly so, in spite of the pathogen, it may be possible to tolerate the presence of the pathogen.

The relatively short contact time of the seedlings with R. similis as compared with field conditions may offer a limitation to studies made in the greenhouse and the temperature tank. In view of the fact that seedling response may be entirely different from mature citrus tree response, it should be pointed out that the effects of Cynem in the mature trees were the same as those observed in the temperature tank tests (14). Field trials are currently in progress to evaluate the long-term effect of some of the more effective treatments on both healthy and decline trees.

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FUNGICIDAL CONTROL OF APPLE BLOTCH, MARSSONINA MALI (P. HENN.) ITO, IN SOUTH KOREA

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Summary

Apple blotch, caused by Marssonina mali (P. Henn.) Ito, has been known in Manchuria, Korea and Japan for a number of years, where it has been a severe foliage disease on susceptible varieties of apples. The disease occurs only on the foliage. It appears to be confined to the Northeast Asiatic area. Folpet, Tuzet, zineb, and Bordeaux mixture plus zinc sulfate appear to afford the greatest protection for the apples.

Apple blotch, caused by Marssonina mali (P. Henn.) Ito, is a serious foliage disease of susceptible apples in South Korea, Manchuria and Japan, where it has been recognized for a number of years (2). No authentic reports have been found of the disease occurring in any other portion of the world. Miss Bensaude (1) reported a leaf spot of apples in the Azores which she thought might be Marssonina mali, but this was never confirmed. A recent letter from the Director of the Commonwealth Mycological Institute, Kew, Surrey states that the disease has not as yet been recorded in England. So far no reference to the fungus having been recorded in the United States has been found in the literature available in South Korea. Thus it appears we may assume that the fungus is confined to the Northeast Asiatic area.

The disease, as it occurs in South Korea, is confined entirely to the foliage and is never found on fruit or twigs. It may completely defoliate susceptible varieties such as the Jonathan or Delicious. Such defoliation results in a heavy fruit fall and a reduction in size and quality (Figs. 2 and 3).

The fungus, one of the Melanconiales, produces shallow acervuli which occur principally on the upper surface of the leaves. The two-celled conidia, which are borne on unbranched conidiophores, are ovoid to oblong in shape and nearly colorless. They are readily scattered by wind, rain and other agents.

The disease makes its first appearance in early May and increases in intensity until it reaches a peak in the latter part of August. By that time susceptible varieties may be completely defoliated. Foliage losses of unsprayed Jonathan trees recorded in the Pomology Section orchard, Suwon, Korea were as follows: 1958, 54%; 1959, 60%; 1960, 73%.

The first symptoms to appear on the leaves are small, light brown, irregular areas which appear most prominently on the upper surface of the leaves. The centers become gray brown and then grayish (Fig. 1) and develop numerous acervuli. The infected areas present a blotchy appearance from whence the disease receives its name.

In 1960 and 1961 a number of fungicides were applied to randomized layouts of 14-year-old Jonathan apple trees in the orchard of the Pomology Section, Institute of Agriculture, Suwon, Korea. In 1960 Bordeaux mixture was applied at 3 1/2 pounds in 100 gallons of water; ferbam (Fermate), zineb(Dithane Z-78) and captan were applied at the rate of 2 pounds in 100 gallons of water and Tuzet (40% thiram, 20% ziram, 20% bis(dimethylthiocarbamylthio)methyl arsine (Urbazid)) was applied at the rate of 0.8 pound in 100 gallons of water.

In 1960 the applications were made with a small power sprayer, capable of developing a pressure of some 200 pounds psi. Applications began May 6 and were continued at approximately 2-week intervals until September 7; 13 applications were made.

The percentage of infection was determined by selecting at random three approximately 1-meter branches from each tree and determining the percentage of infected leaves. A single blotch symptom was considered sufficient to call the leaf infected. The infection data are presented in Table 1.

In 1961 the control trials were continued, with ten treatments applied to a randomized layout of 14-year-old Jonathan apple trees; the layout was similar to that used in the 1960 trials. Of the ten treatments applied, ferbam, zineb, folpet, dodine and glyodin were each applied at the rate of 2 pounds in 100 gallons of water. Mercuric copper and mercuric colloidal copper

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Table 1. Effect of various fungicides on the control of apple blotch in 1960.

Treatment	% infected leaves				
	replication number				
	1	2	3	Sum	Mean
Bordeaux mixture	25.0	27.2	28.2	80.4	26.8
Ferbam	50.6	21.2	27.2	99.0	33.0
Zineb	49.7	29.3	56.8	135.8	45.3
Captan	63.6	52.3	66.3	182.2	70.7
Tuzet	23.0	54.2	49.4	126.6	42.2
Lime-sulfur	70.2	59.3	46.6	176.1	58.7
Potassium	55.8	73.0	84.6	213.4	71.1
Control	63.2	96.4	59.6	219.2	73.1
Totals	401.1	412.9	418.7	1232.7	

Analysis of Variance

Variations due to	Degrees of freedom	Sum of squares	Mean square	F
Blocks	2	18.16	9.08	0.04
Treatment	7	6176.91	882.71	3.98
Error	14	3101.43		
Totals	23	9296.52 [.50]		

LSD at 1% = 52.08
LSD at 5% = 35.11

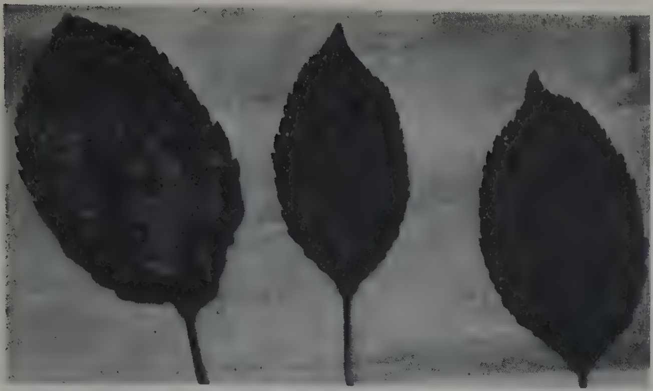
Table 2. Effect of various spray materials on apple brown spot.

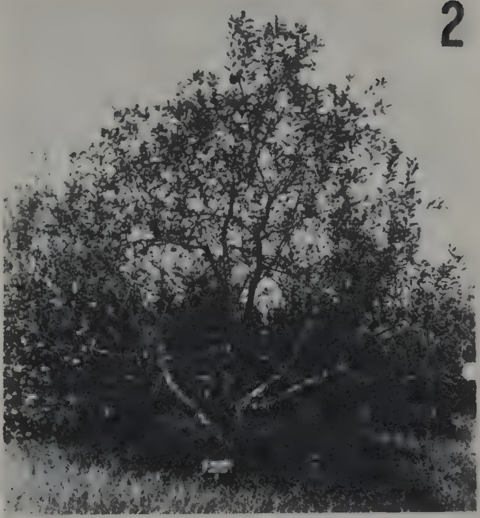
Treatment	% diseased leaves				
	replication number				
	1	2	3	Sum	Mean
Bordeaux	1.9	1.2	1.1	4.2	1.4
Ferbam	3.3	2.7	4.0	10.0	3.3
Zineb	2.0	2.4	2.4	6.8	2.3
Tuzet	6.5	5.5	2.8	14.8	4.9
Folpet	1.2	5.1	5.3	11.6	3.9
Dodine (Malprex)	3.5	2.7	1.1	7.3	2.4
Mercuric copper	11.1	1.3	2.9	15.3	5.1
Glyodin	23.9	54.6	2.8	81.3	27.1
Colloidal mercuric copper	42.6	37.3	62.3	142.2	47.4
Control	45.6	59.4	49.0	154.2	51.4
Totals	141.8	172.2	133.7	447.7	

Analysis of Variance

Variations due to	Degrees of freedom	Sum of squares	Mean square	F
Blocks	2	89.38	44.69	0.358
Treatments	9	9953.14	1105.90	8.88 **
Error	18	2241.18	124.5	
Totals	29	12,283.70		

LSD at 1% = 26.24
LSD at 5% = 19.13





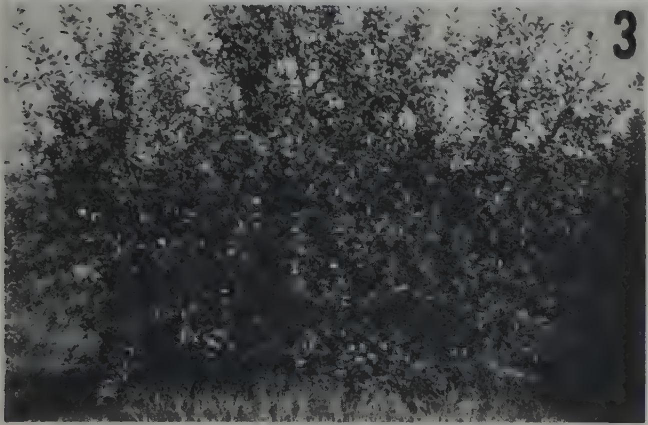


FIGURE 1. Apple blotch on leaves of Jonathan apples. Suwon, Korea 1960.

FIGURE 2. A 14-year-old Jonathan apple tree unsprayed. Photograph taken in August 1960 at Suwon, Korea.

FIGURE 3. A 14-year-old Jonathan apple tree sprayed with ferbam. Photograph taken in 1960 at Suwon, Korea.

were applied at the rate of 2 1/2 pounds in 100 gallons of water, Tuzet at 0.8 pound in 100 gallons of water, and Bordeaux mixture at 2-12-100 with the addition of zinc sulfate added at the rate of 40 grams to each 20 litres (5.28 gallons) of Bordeaux mixture. Four grams of malathion were added to each gallon of spray to protect against insects.

Applications were made on May 20, June 9 and 22, July 4, 15 and 25, August 5, 17 and 28 and September 9. The percentage of blotch was determined by selecting at random three approximately 1-meter branches on each tree and counting the leaves on each branch. The rate of infection became progressively more severe from the middle of May until the harvest period in September. Figure 2 illustrates an unsprayed tree in August and Figure 3 a tree that was sprayed with ferbam. Both photographs were taken on the same day. The data from the 1961 trials are presented in Table 2.

DISCUSSION

In 1961 zinc sulfate was added to the Bordeaux mixture to prevent foliage injury and as a result the Bordeaux mixture-zinc sulfate combination was the best fungicide for the control of the blotch. However, within the limits of the 1961 trials there was no significance between the controls of Bordeaux mixture-zinc sulfate, ferbam, zineb, Tuzet, folpet, dodine or mercuric copper. The results of the 2 years' trials with Bordeaux mixture, ferbam, zineb and Tuzet were very similar. From the results of the 2 years' trials it appears reasonably safe to recommend Bordeaux mixture-zinc sulfate, ferbam, zineb, folpet, captan, Tuzet or mercuric copper for control of apple blotch. The availability and price of the fungicide would be the deciding factor. Bordeaux mixture is readily available, is among the cheapest on the local markets, and is well known to the Korean orchardist. It is likely to be the most commonly used fungicide for orchard disease control.

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REACTIONS OF WINTER OATS TO SOIL-BORNE MOSAIC[1]

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McKinney (4) indicates that the soil-borne mosaic diseases of oats Marmor terrestre var. typicum and M. terrestre var. oculatum were first observed in the United States at Auburn, Alabama in 1943. Since 1943, soil-borne winter oat mosaic has been observed in numerous other areas.

The disease was suspected of being present at the Plant Industry Station South Farm, Beltsville, Maryland in 1959, and its presence there was proved in 1961. Bruehl and Damsteegt (2) reported in 1961 that the disease was present in winter oats in western Washington.

From these observations, plus reports received earlier from Atkinson (1), McKinney, et al. (5), Moseman, Gore, and McKinney (6), and Earhart and Moseman (3) indicating that the disease was present in the Carolinas, Georgia, and Alabama, it becomes increasingly evident that soil-borne mosaic is much more widely present that previously realized.

The actual amount of damage caused by soil-borne mosaic has not been determined, but potential losses may be close to 100%, as in extreme cases the plants fail to produce panicles. No variety now available is immune from this disease and at best the oats being grown are only tolerant. Therefore, reaction to soil-borne mosaic must be given more serious consideration in new oats for fall seeding than in the past.

MATERIALS AND METHODS

With a few exceptions, from 1953 to 1961 inclusive, uniform oat mosaic nurseries were grown annually in North Carolina, South Carolina, and Georgia. The station farms on which the nurseries were located differed somewhat from year to year in North and South Carolina, but in Georgia all nurseries were conducted on the Georgia Experiment Station farm near Griffin.

In all nurseries the seeding plan remained uniform. Duplicate series of short rows, 3 to 5 feet in length, of all entries were sown. A susceptible check variety, usually Letoria, was sown every fifth row.

The number of entries, including checks, differed in different years but ranged as high as 500 to 700 in each of several recent years. Consequently, about 30,000 rows were sown and the plants observed from 1953 to 1961.

The seeding plans and lists were prepared and the seed of the strains included was packeted at Plant Industry Station. The observations on the reaction of the entries to mosaic have been reported by those under whose supervision the test nurseries were conducted. All data received from Georgia were recorded by U. R. Gore. The nurseries in North Carolina since 1953 were conducted by T. T. Hebert. J. G. Moseman recorded the data in that State in 1953. In both Georgia and North Carolina, all data were recorded in percents of infection. In South Carolina, Robert W. Earhart recorded the data from 1955 to 1959 and W. P. Byrd in 1960 and 1961. Observations at Plant Industry Station in 1961 were recorded by F. A. Coffman. At Clemson, infection types were recorded as from 1.0 to 10.0, whereas infection types were recorded at Beltsville as from 0.0 to 4.0. At both stations, the lower figure indicates the least infection and the higher figure the completely susceptible type.

RESULTS OBTAINED

In Table 1 data are included on a number of the older varieties, plus all winter oat varieties named and released from 1953-1961, inclusive. In addition, data were recorded on all entries in regional uniform winter oat yield and hardiness nurseries. Thus, data are actually

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Agricultural Experiment Stations of Georgia, North Carolina, and South Carolina.

[2] Research Agronomist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture; Professor of Plant Pathology, North Carolina Agricultural Experiment Station; Agronomist, Georgia Agricultural Experiment Station; and Associate Agronomist and Associate Professor of Agronomy, Clemson College, South Carolina, respectively. The writers are indebted to Robert W. Earhart for data obtained at Clemson, South Carolina from 1955 to 1959, inclusive, and to John G. Moseman for data obtained in North Carolina in 1953.

Table 1. Summary data for 9 years on reaction to soil-borne mosaic of named winter oats, in comparison with Arlington check grown in two or more nurseries from 1953 to 1961, inclusive.

Rank in tolerance N. C. & Ga. Av.	C. I. no.	Variety or selection	Nurseries N. C. & Ga. (no.)	Dev. from check (av. %)	Maximum infections recorded		
					N. C. & Ga. (%)	S. C.: type[a]	Md. type[b]
1	7603	Excel	2	-6.3	20	5.0	1.0
2	7622	Early Ballard	2	-5.0	20	1.5	1.0
3	6980	Ballard	10	-3.5	30	3.0	0.0
4	4599	Atlantic	3	-1.0	5	---	---
5	3168	Fulwin	12	-0.3	40	5.5	1.5
6	4657	Arlington (check)	16	+0.0[c]	30	4.9	1.0
7	3170	Forkedeer	5	+1.4	10	---	---
8	7229	Moregrain	9	+1.7	40	7.0	1.0
9	7125	Victorgrain 48-93	11	+3.9	40	6.5	2.0
10	6588	Floriland	13	+4.0	40	7.0	0.0
11	5107	LeConte	15	+4.1	50	3.5	2.0
12	6903	Norline	6	+4.2	40	3.0	2.0
13	5872	Nortex (Stoneville strain)	4	+4.3	20	---	---
14	7509	Sumter	2	+5.0	40	6.5	0.0
15	6902	Stanton (Oklahoma strain)	3	+5.0	20	5.0	---
16	5106	Cimarron	11	+5.9	50	5.0	---
17	6977	Midsouth	8	+5.9	50	7.0	---
18	7417	Fairfax	6	+6.7	50	7.0	1.0
19	2499	Pentagon	14	+6.8	50	6.5	2.5
20	7413	Roanoke	6	+8.8	50	5.5	1.0
21	3424	Wintok	14	+11.4	50	6.0	1.0
22	708	Fulghum	12	+11.6	50	8.0	2.0
23	5207	Southland	9	+11.9	60	7.5	---
24	3923	DeSoto	5	+12.4	40	2.6	---
25	1815	Appler	12	+12.9	80	7.5	1.0
26	3531	Fultex	11	+15.4	40	6.5	---
27	5850	Arkwin	4	+17.4	50	---	---
28	6600	Sunland	3	+18.3	50	5.5	---
29	2042	Lee	12	+18.7	80	6.0	1.0
30	7404	Arkwin (reselection)	6	+18.8	70	4.3	1.0
31	5849	Wintok (selection)	2	+20.0	40	---	---
32	4653	Delair	8	+20.9	100	9.0	---
33	7513	Carolee	4	+22.5	90	6.0	1.0
34	6571	Bronco	12	+23.3	80	5.5	---
35	2505	Hairy Culberson	13	+25.4	90	7.5	3.0
36	5924	Seminole	5	+27.5	90	5.5	---
37	6718	Lemont	8	+29.0	100	---	---
38	3296	Winter Turf	12	+29.1	100	7.5	1.0
39	7420	Florad	4	+31.3	90	9.5	4.0
40	4600	Coy	8	+34.3	100	5.0	---
41	6572	Dubois	13	+35.5	100	4.7	2.0
42	7339	Radar 1	7	+43.6	100	8.5	1.0
43	3855	Stanton 1	5	+44.4	100	3.0	---
44	7340	Radar 2	7	+49.3	100	9.5	1.0
45	5371	Alamo	13	+50.2	100	9.0	2.0
46	3392	Letoria[d]	3	+50.7	100	---	---
47	5364	Nysel	10	+52.5	100	7.0	3.5
48	4660	Mustang	12	+53.1	100	8.0	---
49	7425	Alamo Miss. H. V. R.	4	+53.8	100	9.5	0.0
50	7426	Floriland (irradiated)	3	+61.7	100	9.5	4.0
51	947	Tech	12	+68.5	100	9.5	3.0
52	5118	Colwin	3	+74.0	100	---	---

[a] Types at Clemson, S. C., ranged from 1.0 to 10.0 in 1 to 7 years.

[b] Types at Beltsville, Md., ranged from 0-4.0 in 1 year only.

[c] Average infection for Arlington check was 11.4 percent.

[d] Used as susceptible check in all nurseries and 3 nurseries reported on for Letoria sown as a regular entry only.

available on well over 300 strains of winter oats but space does not permit including all these data. Data on strains designated only by C. I. numbers are available only on request. In addition to the oats mentioned, several hundred advanced breeding lines have been tested in these nurseries. Some of these may later be assigned C. I. numbers.

Data in Table 1 indicate the calculated average percents of infection recorded in North Carolina and Georgia. Arlington, C. I. 4657, was included in all nurseries grown, and the figures shown are for average deviation from check in percent of infection based on Arlington in the same nurseries. If an entry had a higher average infection than Arlington in any given nursery, it was given as a plus ("+") reading. If its average infection percentage was below that of Arlington, it was minus ("-"). The figures and deviations shown were determined by adding the individual nursery readings, with due consideration as to whether readings were "+" or "-". The sum for all years divided by the total number of nursery reports included gave the figure. Data are also included as to the maximum percent of infection recorded in any year in either Georgia or North Carolina, together with maximum infection-type ratings from South Carolina and Maryland. This additional information is included primarily as supporting evidence.

CONCLUSIONS AND SUMMARY

Soil-borne mosaic of oats has caused considerable loss in the past decade. No variety available is immune from this disease, and it is questionable whether any oat should be sown on soil known to be infested. Several new oats have considerable tolerance; others are highly susceptible. With such a wide range of resistance available, it is not necessary to seed highly susceptible oats in any area.

Data available indicate a wide range in tolerance to soil-borne mosaic. The most highly tolerant varieties appear to be Excel, Ballard, Early Ballard, Atlantic, Fulwin, and Arlington. Other tolerant varieties are Forkedeer, Moregrain, Victorgrain 48-93, Floriland, LeConte, Norline, Nortex, Sumter, and the Stanton (Oklahoma strain). Fairfax and Roanoke, although tolerant, are somewhat less tolerant than are those already listed. Almost no other recently released oat variety can be considered as having much tolerance. The data included may be useful in making decisions as to the variety to grow or as to the variety to use as a parent in crosses to produce winter oats that will tolerate soil-borne mosaic. Data obtained indicate that the resistance found in Ballard is inherited in the hybrid progeny (unpublished data, Coffman).

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EVALUATION OF TREATMENTS FOR CONTROL OF SOIL-BORNE PESTS IN CELERY SEEDBEDS[1]

J. F. Darby, C. E. Dieter, and G. J. Rau[2]

Abstract

Several pesticide drenches were evaluated as possible substitutes for methyl bromide and chloropicrin confined under plastic film. Included were 3,5-dimethyl-tetrahydro-1,3,5, 2H-thiadiazine-2-thione, sodium methyldithiocarbamate, and mixtures of allyl alcohol with formalin, urea-formaldehyde, emulsible ethylene dibromide and emulsible dichloropropene-dichloropropanes. These drenches were found to be satisfactory preplanting treatments for the control of soil-borne pests in celery seedbeds, but they were all less effective than methyl bromide or chloropicrin.

Duplicate soil samples were collected for nematode analysis from the plots, but processed and counted in different laboratories. A high degree of agreement demonstrated the reliability of nematode counts in evaluating pesticides.

INTRODUCTION

Fumigation of the soil with methyl bromide before planting celery seedbeds has been recommended by this station since 1953 (4). The results have been excellent, but the cost and inconvenience of applying and reclaiming the plastic film are definite disadvantages. This is one of a series of tests begun in 1956 to determine the efficacy of using drenches to control soil-borne pests in celery seedbeds in lieu of methyl bromide under plastic film. In the central Florida area, drenching is a convenient and economical method of treating celery seedbeds because an abundant water supply from artesian wells is available, and celery growers have large watering carts easily adapted for applying drenches.

MATERIALS AND METHODS

The area used for this experiment has been under cultivation for at least 50 years and has a history of high populations of nematodes, fungi, and weeds. The soil (Leon fine sand) was cultivated thoroughly, fertilized, and each seedbed was kept moist by an underground tile irrigation system for 2 weeks before treatment.

Methyl bromide (Dowfume MC-2 methyl bromide with 2% chloropicrin) was released under vinyl plastic film from 1-pound cans by means of an applicator with saran tubing. Chloropicrin was injected 6 inches deep on alternating 12-inch centers by means of a hand injector. The treated areas were then covered with vinyl plastic film. The film was allowed to remain in place for 48 hours. The plots were 10 feet long and 4 feet wide and were replicated four times.

The ingredients in the drenches, allyl alcohol, 83% emulsible ethylene dibromide (EDB as Dowfume W-85), 86% emulsible 1,3-dichloropropene-1,2-dichloropropane (D-D mixture as Shell D-D), a mixture containing 25% urea and 60% formaldehyde (U. F. Concentrate-85), and formalin (40% formaldehyde) were mixed in the proportions listed in Table 1 prior to adding to water and applying as drenches in approximately 1/3 inch of water. The total amount of nitrogen applied to each plot was equalized by adding low-biuret urea to those plots not receiving U. F. Concentrate-85. The following materials were applied as drenches in approximately 1/3 inch of water: 3,5-dimethyl-tetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT as Mylone 85W), and 32.7% sodium methyldithiocarbamate (SMDC as Vapam). The soil temperature 3 inches deep at the time of treatment on September 13 was 94°F. A thermograph was installed in one of the plots to record the air temperature 2 feet above the soil surface and the soil temperature at a 6-inch depth. As winter approached, the soil temperature dropped to a minimum of 67° and the air temperature to 64°.

One week after treatment the plots were aerated by chopping to a depth of 3 inches with garden rakes. The surface soil was high in soluble salts at the end of the normal waiting period of 2 weeks. Planting of the celery was delayed until 27 days after treatment to permit leaching

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Table 1. Results of treatments for control of soil-borne pests of celery seedbeds. All data are averages of four replicates, and treatments are listed in descending order of effectiveness.

Treatment and quantity/acre	Weed control rating[a]	Percentage nematode reductions[b] Series 1	Series 2	Yield rating[a]
1. Methyl bromide, 871 lb	5.0	88.4	87.6	4.2
2. Chloropicrin, 951 lb	4.2	86.6	90.0	4.0
3. Allyl alcohol, 40 gal. EDB (83% emulsible), 6 gal. U. F. Concentrate-85, 67 gal.	4.6	76.1	82.4	3.9
4. Allyl alcohol, 40 gal. EDB (83% emulsible), 6 gal. formaldehyde (40%), 100 gal.	4.5	76.4	88.2	3.8
5. DMTT, 85W, 300 lb	4.1	77.7	88.7	3.6
6. Allyl alcohol, 40 gal. D-D (86% emulsible), 25 gal.	4.6	61.3	73.0	4.0
7. Allyl alcohol, 40 gal. D-D (86% emulsible), 25 gal. formaldehyde (40%), 100 gal.	4.6	77.3	60.0	3.7
8. Allyl alcohol, 25 gal. D-D (86% emulsible), 25 gal.	4.6	65.2	57.4	4.2
9. SMDC, 75 gal.	4.2	68.8	56.2	4.5
10. Allyl alcohol, 25 gal. D-D (86% emulsible), 25 gal. formaldehyde (40%), 100 gal.	4.2	59.7	72.5	3.8
11. SMDC, 100 gal.	4.5	59.7	50.3	3.9
12. Untreated	2.2	45.4	47.3	1.4
LSD 5%	0.7	25.0	33.7	0.5
1%	1.0	33.5	45.1	0.7

[a] Weed control and yield ratings are based on 0 to 5 scales with 5 indicating complete weed control and maximum yield, respectively.

[b] 82 days after treatment in relation to pretreatment nematode populations.

of the soluble salts to a safe level before seeding.

Methods of Evaluation: Weed control ratings were made on a scale ranging from 0, indicating no control, to 5, maximum control. No effort was made to identify the weeds and grasses.

Nematode populations were determined by collecting duplicate 500-g samples of soil from the surface to a depth of 8 inches. The soil samples were taken before treatment and 15, 82, 111, and 150 days after treatment. One set (Series 1) was processed and counted in the laboratory at the Central Florida Experiment Station, Sanford, Florida by Rau and the other (Series 2) in The Dow Chemical Company laboratory at Winter Park, Florida by Dieter. The modified Baermann funnel technique of Christie and Perry (1) was employed in each laboratory for the recovery of the nematodes. The pretreatment population was used as a basis for calculating the percentage reduction in nematodes. Angular transformation was used in the analysis.

Yield ratings were made by taking the weight of usable celery transplants from 1 foot^2 of each plot and converting it to a scale ranging from 0 to 5, with 5 being the maximum yield. This was averaged with a visual rating (0-5 scale) made over the entire 40-foot^2 plot and presented as one rating in Table 1.

Disease control ratings are not presented because of a lack of measurable disease present in the chemically treated plots. The plants in the untreated plots were moderately infected with *Pythium*, *Rhizoctonia*, and *Fusarium* and this was reflected in a reduced yield.

The rank of each treatment in weed control, nematode control, and yield were averaged and listed in Table 1 in descending order of performance.

RESULTS AND DISCUSSION

The parasitic nematode population collected included mostly sting, *Belonolaimus longicaudatus* Rau, stubby-root, *Trichodorus christiei* Allen, sheath, *Hemicycliophora parvana* Tarjan, and lance, *Hoplolaimus tylenchiformis* (Daday) Andrassy. The nematode data from samples collected 15, 111, and 150 days after treatment are not presented because all the treatments controlled the nematodes 15 days after treatment while at 111 and 150 days after treatment only the methyl bromide and chloropicrin treated plots had significantly lower nematode populations than the untreated. Most of the nematodes present after 82 days in the treated plots consisted of stubby-root with a few lance nematodes, whereas in the untreated plots the number of stubby-root nematodes remained approximately the same while there was a reduction in the total parasitic nematode population as winter approached (Table 1).

In the mixtures, the allyl alcohol was incorporated as an herbicide, the EDB or D-D as a nematocide, and the formaldehyde or U. F. Concentrate-85 as a fungicide. The addition of formaldehyde to allyl alcohol and EDB did not significantly increase the effectiveness of the treatment under the moderate disease conditions of this test. In previous tests, 25 gal. /acre of allyl alcohol has not been consistently effective in weed control. In these tests, 40 gal. /acre of allyl alcohol was slightly more effective than 25.

A high concentration of soluble salts at the surface of the soil during dry weather is common in this area because of the use of an underground tile system for irrigation with artesian well water. This water has a salt content of between 300 and 500 ppm. The yield and the reduction in nematodes was greater from those plots treated with 75 gal. /acre of SMDC than from those treated with 100 gal. /acre of SMDC (Table 1). This was attributed partly to the phytotoxicity of the higher rate which was accentuated by the high concentration of soluble salts and partly to the fact that at 150 days after treatment there were over twice as many stubby-root nematodes in those plots treated with the higher concentration of SMDC. The re-establishment of stubby-root nematodes following fumigation is common in this area. Perry (3) attributed this to the destruction of natural enemies, including predaceous nematodes, by fumigants.

A comparison of the nematode counts made from duplicate soil samples taken from the same plots, but processed and counted in different laboratories, showed a high degree of agreement and made the nematode data more reliable.

Chemical drenches were satisfactory substitutes for methyl bromide and chloropicrin under plastic film, but they were slightly less effective even under the favorable weather conditions encountered during this test. Darby (2) observed that heavy rainfall soon after application of drenches diluted the chemicals below effective concentrations. Extremely high soil temperatures (95° and over) in some tests allowed the pesticide fumes to escape too rapidly, whereas methyl bromide or chloropicrin confined by plastic film was consistently effective. Further evidence is that none of the drenches controlled nutgrass (Cyperus esculentus) in this or in previous tests as well as did methyl bromide or chloropicrin.

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DOES THE CORN LEAF BLIGHT FUNGUS SURVIVE WISCONSIN WINTERS?[1]

Paul E. Hoppe[2]

Northern leaf blight, caused by Helminthosporium turcicum Pass., has become a serious corn disease problem in Wisconsin in some years. This disease, unknown to the State prior to 1952 when a heavy epidemic developed, has since occurred heavily in some years and very sparingly, if at all, in others. Studies to determine possible reasons for these sporadic outbreaks were begun in 1957.

HISTORY OF THE DISEASE IN WISCONSIN

The original epidemic of leaf blight in 1952 developed over wide areas, particularly in southern and western parts of the State. Extensive damage to the corn crop was limited mainly to a west-central area where many fields were prematurely killed. Initial infections in this region are believed to have occurred the last week of July. In the 5-year period from 1953 through 1957, leaf blight from natural sources of infection rarely was observed in Wisconsin. A second and more widespread epidemic occurred in 1958 when primary infections developed in September -- too late for significant damage to the crop. The most severe and damaging outbreak of northern leaf blight yet experienced in Wisconsin occurred in 1959. Primary infections were seen in late July, and by mid-September (before killing frosts) essentially every corn field in the State was brown and dried up as a result of the disease. The subsequent stalk rot and stalkbreaking was the worst since the days of open-pollinated corn. Leaf blight again developed over the entire State in 1960. Primary infections, however, were later than in 1959 and less damage to the crop resulted. In 1961 the disease was severe only in east-central and northeastern Wisconsin.

ARTIFICIALLY INDUCED EPIDEMICS

Artificial inoculations with H. turcicum were begun at the Wisconsin Agricultural Experiment Station in 1953 and have been continued each year since. Heavy epidemics of leaf blight developed each year regardless of wide differences in weather conditions after the inoculations. In the years when natural epidemics of the disease occurred, blight development in the inoculated plots reached an advanced stage weeks before the primary infections from natural sources were observed.

The inoculation technique involves only a single, but thorough, dusting of the plants with finely ground diseased leaf material saved from the preceding year. The diseased leaves are stored through the winter in the Seeds Building, at 58° F, and ground to a fine powder shortly before inoculation time. Inoculations are made early in the morning when the plants are covered with dew and are about knee-high. Primary infections usually develop on lower leaves as well as in the whorl. Disease development from artificial inoculation is similar to that which follows natural infections. The technique is equally effective for seedling inoculation in the greenhouse.

WINTER SURVIVAL STUDY

Study of the winter survival of H. turcicum under outdoor or natural conditions of exposure in Wisconsin was prompted by the following consideration. If the environment always favors leaf blight, as indicated by the results from artificial inoculations, the natural outbreaks which have occurred sporadically in Wisconsin since 1952 must have been governed by the presence or absence of inoculum.

The survival studies were begun in the winter of 1957-58 and continued for 3 years. A description of the experiments follows.

1957-58: Leaves heavily infected with H. turcicum were collected in the fall of 1957 and placed in an onion bag which then was staked to the ground in an open field. Beginning in November, leaf samples were taken monthly and tested for disease potential on corn seedlings

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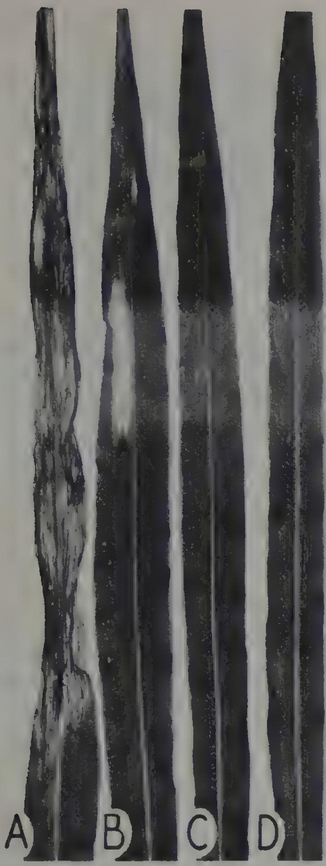

FIGURE 1. Northern leaf blight in corn seedling leaves following inoculations with finely ground leaf material from heavily infected leaves exposed the preceding winter to varying environments: A -- Leaves kept in Seeds Building at 58° F. B -- Exposed outdoors in suspended onion bag. C -- Exposed outdoors in onion bag in contact with soil. D -- Non-inoculated control.

in the greenhouse by the ground-leaf inoculation technique.

Blight development from the winter-exposed leaves was at a high level throughout the early winter, but thereafter diminished gradually until by April it no longer was possible to induce seedling lesions. Blight from leaves kept in the Seeds Building developed at a high level at this time.

1958-59: Three conditions of exposure were made in addition to exposure in bags on the soil in an open field: 1) leaves covered with about an inch of Miami silt loam, 2) leaves suspended above soil in an onion bag, and 3) leaves similarly suspended but protected in waterproof wrapping. Monthly survival tests were made for all conditions of exposure except where leaves were covered with soil, in which case a test was made only in May and with no blight lesions developing.

The results from leaves in the bag laid on the soil were the same as those in the preceding year. Many lesions developed in the early winter, but none in the tests made in April.

Somewhat different results were observed in the tests with leaves that had been suspended above ground. An occasional blight lesion developed from both wrapped and unprotected leaves as late as May, when the tests were concluded.

1959-60: Exposure conditions were like those of the previous winter except that no leaves were covered with soil. Survival tests were made only in May with results like those of the year before -- no disease from leaves in contact with soil throughout the winter and an occasional lesion from those suspended above the ground (Fig. 1).

1960-61: A more natural condition of exposure was tested this winter. All plants in an area of approximately 30 x 150 feet in a heavily blighted field were cut in October and left lying on the ground. Plants also were piled to a depth of 12 to 14 inches for a slightly less severe winter exposure. Greenhouse inoculations made in early May 1961 indicated no survival of H. turcicum in the leaves of either scattered plants or those that had been left in piles. Disease level from leaves kept in the Seeds Building, as usual, was high.

DISCUSSION AND CONCLUSIONS

Robert[3] states that H. turcicum overwinters inside infected corn leaves left in the field. These experiments indicate but a very limited survival of the fungus through Wisconsin winters and then only when infected leaves are exposed under abnormal conditions. Under natural conditions of exposure, with leaves in contact with soil, the mortality was complete. These results strongly suggest that the outbreaks of leaf blight that occurred sporadically in Wisconsin over the last 9 years must have been initiated by H. turcicum spore showers, blown into the State, presumably from the South. That such showers have occurred here was amply demonstrated in the first natural epidemic, in 1952.

It is not to imply that strains of the corn race of H. turcicum may not develop an adaptability for overwintering in northern States. The genus Helminthosporium is noted for its intraspecific variability, but whether such strains may develop remains to be seen.

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STING NEMATODE ON GRAPE CUTTINGS IN FLORIDA[1]

N. C. Schenck, J. A. Mortensen, and L. H. Stover[2]

Summary

In Florida, Belonolaimus longicaudatus was associated with grape cuttings which failed to develop normally. Roots from affected cuttings frequently had swollen tips and a characteristic "stubby" appearance. Shoots from affected cuttings wilted and became necrotic. This is the first report of B. longicaudatus on grape.

In the spring of 1961, hardwood grape cuttings at several locations in the nursery of the Watermelon and Grape Investigations Laboratory did not sprout normally (Fig. 1). Some cuttings in the affected areas failed to develop new shoots, others sprouted normally but later wilted or bore leaves with necrotic margins. After the first shoot had wilted and died another bud frequently sprouted, but the resultant shoot also wilted and became necrotic. Roots of affected plants were commonly discolored and necrotic. Others had a characteristic "stubby" appearance. They were coarse and much shorter than normal (2 to 4 mm), and frequently had swollen tips. In some instances no roots developed.

Soil samples were taken from the rooting zone of both affected and healthy-appearing cuttings. The number of nematodes in these samples was determined, using the inverted flask technique described by Chapman (1). Sting nematodes (Belonolaimus longicaudatus Rau)[3] occurred in all soil samples from the rooting zone of affected cuttings, ranging from 15 to 60 per 125 g of soil. B. longicaudatus occurred in two of the three samples from healthy-appearing plants but averaged only two nematodes per 125 g of soil. Other plant parasitic nematodes associated with affected cuttings were species of Trichodorus, Pratylenchus, and Criconemoides, but these nematodes did not occur in all samples and were recovered in small numbers only.

The 1961 grape cutting nursery was situated on land cropped to tomatoes and cowpeas in the spring of 1959. Florida beggar weed (Desmodium tortuosum) also was present in the nursery area. All of these species are listed by Christie (2) as suitable hosts for increasing populations of B. longicaudatus.

Twenty-four grape varieties and 31 experimental grape selections were planted in the cutting nursery. Although most entries in the nursery were not replicated, several varieties and selections were planted in sufficient numbers to minimize the possibility of their being escapes from soil infested with sting nematodes. The variety Dogridge (Vitis champini), reported as nematode resistant (3), and Florida Selections 449 and 380 were some of the most vigorous entries in the nursery. Less than 50% of the cuttings of most entries survived. From 100 original cuttings of R. W. Munson and Demko 14, less than 10% survived by June 28.

This is the first report of B. longicaudatus on grape and the first report from Florida implicating a nematode in a disorder of grape. Because of the distribution pattern of the diseased plants in the nursery and the appearance of the roots of affected plants, the sting nematode is believed to be the primary cause of this disorder. However, both Fusarium and Rhizoctonia species were isolated from discolored roots of affected plants and also may have been involved.

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[3] Thanks are expressed to V. G. Perry for the species determination.

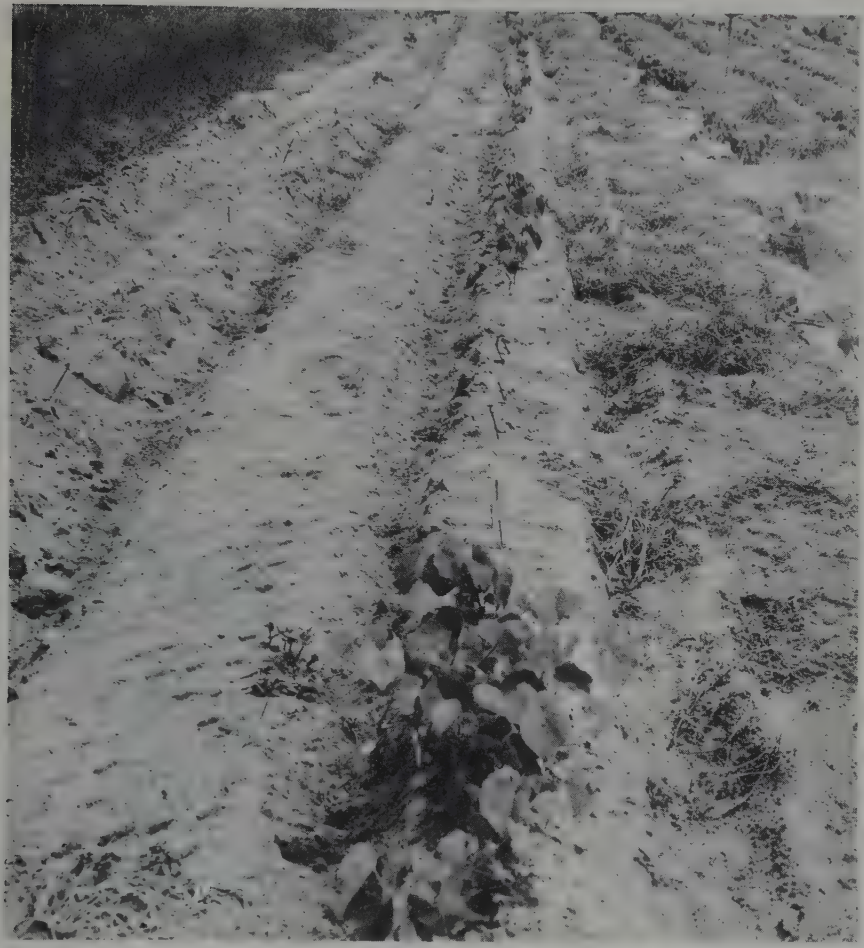

FIGURE 1. Failure of hardwood grape cuttings in nursery. Large numbers of sting nematodes were recovered from soil in affected areas. (Photograph by R. C. Orr)

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WATERMELON AND GRAPE INVESTIGATIONS LABORATORY, FLORIDA AGRICULTURAL EXPERIMENT STATION, LEESBURG, FLORIDA

BROOMING AND GALLING OF SHAGBARK HICKORY IN ARKANSAS[1]

Charles L. Wilson[2]

Extensive brooming and galling of the branches and stem of a 5-acre stand of mature shagbark hickory (Carya ovata) near Kingston, Arkansas (Carroll County) was studied. Isolated trees that were similarly affected were found in other northern areas of the State. The brooms were similar to those described by Stewart (10) except there appeared to be more enlargement of the branch tissue associated with them (Fig. 4). Also, galls associated with the brooming were found in the main stem of some trees (Fig. 2). Few reports of similar malformations on hickory were found. Gronemann (5) states that witches' brooms of unknown cause occur on Carya alba in the Chicago area. Hepting and Toole (6) report a similar condition on Carya sp. in North Carolina. Stewart (10) describes witches' brooms on Carya ovata in New York. Microstroma juglandis (Bereng.) Sacc. was found infecting consistently the leaves produced by the brooms and little infection was found on the leaves from adjacent branches that were not broomed. He concluded that the witches' brooms were probably incited by M. juglandis. Buhr (2) reports that small vesicular outgrowths are formed on the leaves of young walnuts (Juglans regia) infected by M. juglandis, and a catkin blight is produced on pecan (C. illinoensis) by M. juglandis var. robustum (7).

Witches' brooms have been noted on other members of the Juglandaceae. Japanese walnut (Juglans cordiformis var ailantifolia) in the eastern and southern United States and butternut (Juglans cinerea) and black walnut (J. nigra) in the eastern United States sometimes produce witches' brooms (8, 9). This condition is believed to be caused by a virus, since it is transmissible by grafting (8). A rosette disease of pecan (Carya illinoensis), associated with zinc deficiency, may result in malformation of the branches (4).

OBSERVATIONS

The incipient stages in the development of the witches' broom and galling can first be seen as a callus overgrowth at the base of branches (Fig. 1). The branch is inhibited in its apical extension and extension from lateral buds is stimulated (Fig. 6). The callus overgrowth produces additional buds that elongate into short shoots. The branch tissue to which the broom is attached enlarges in subsequent years and produces a gall. A number of brooms may develop on one branch and the galled tissue will become confluent, producing a greatly enlarged branch with numerous witches' brooms (Fig. 4). Branches may become three to four times their normal diameter (Fig. 4). The enlargement of stem tissue (galling) is closely associated with the witches' brooms. When galls develop on the main stem, branches extend from the galls that are broomed (Fig. 2). This distinguished these stem galls from overgrowths that may be associated with cankers produced by Poria spiculosa Campbell & Davidson (3) or tumors produced by Phomopsis sp. (1).

The malformations may be present on all the branches of an individual tree (Fig. 3). They appear to cause little overall detriment to the tree except in cases where galls develop in the main stem. Trees were observed that had broken over at galls on the main stem (Fig. 5). The affected tissue itself is stimulated in its diameter growth. There is a two- to three-fold increase in the width of annual rings in the galled areas; also, the phloem tissue is enlarged.

As indicated by the increase in diameter of annual rings, some of the witches' brooms and subtending galls were over 10 years old. Each year new buds are produced from the callus growth on the branch from which short foliage-bearing shoots develop. A number of the previously formed shoots also die each year. The foliage from the witches' brooms is initially normal but held in a rosette pattern due to the arrangement of the shoots.

There is a progressive decay of the older galls which continue to proliferate new tissue from which new buds are produced. Carpenter ants often inhabit the decayed portions of older galls but no insects were found associated with young witches' brooms or galls.

Isolations from the galled tissue and shoots of the witches' brooms failed to yield any organisms consistently. M. juglandis was found consistently fruiting on the foliage from brooms, whereas foliage from adjacent normal branches were free from this organism. Early in June the undersurface of leaves from the brooms turns white from the sporulation of M. juglandis. Black lesions with this fungus fruiting on them are produced on the petiole and commonly the

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[1] Published with the approval of the Director of the Arkansas Agricultural Experiment Station.
[2] Assistant Professor, Department of Plant Pathology.

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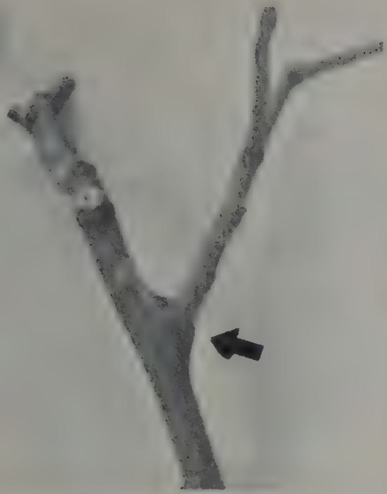

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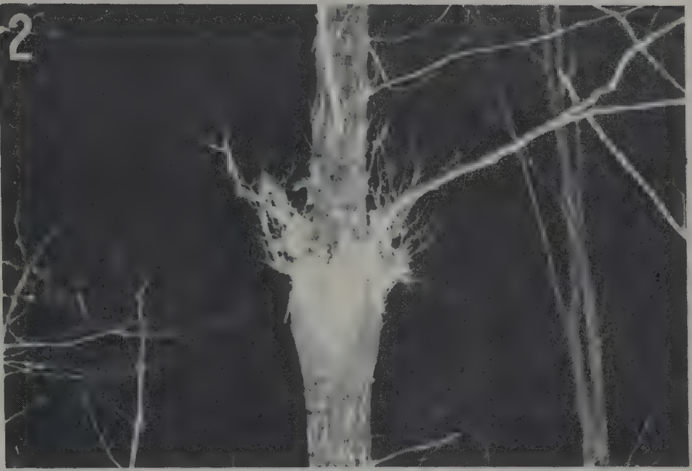

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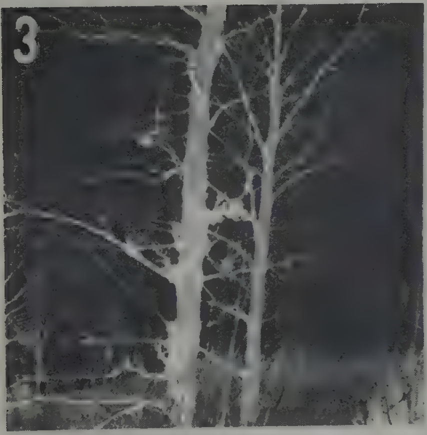

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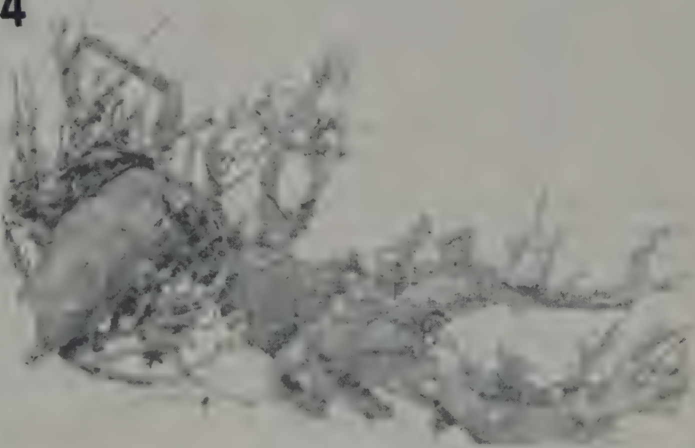

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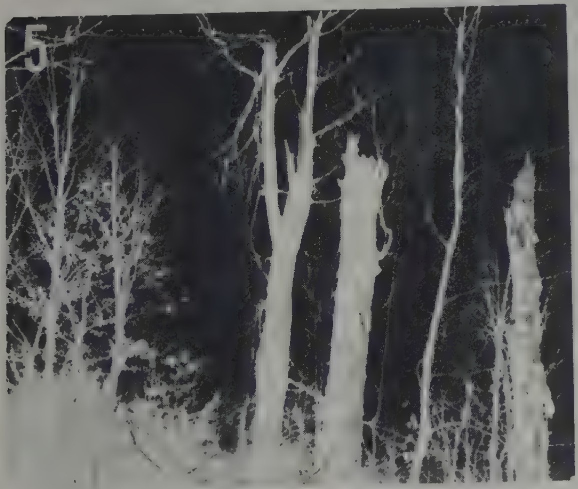

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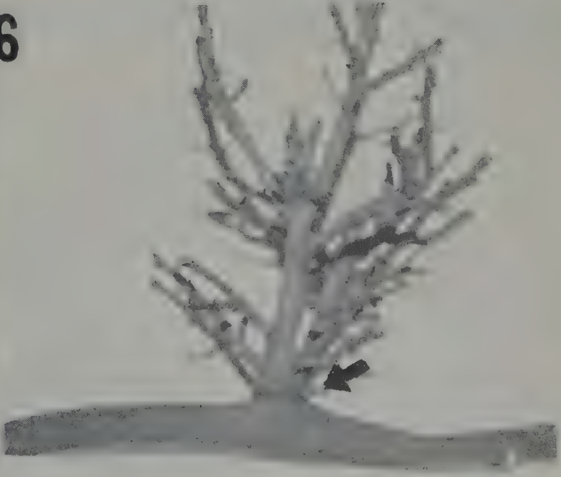

FIGURE 1. Enlargement at the base of a branch (arrow).
FIGURE 2. Gall on the main stem of Carya ovata with issuing brooms.
FIGURE 3. Carya ovata with numerous branches broomed.
FIGURE 4. A branch that has become greatly enlarged and is producing numerous brooms.
FIGURE 5. Carya ovata that have broken over at galls on the main stem.
FIGURE 6. A witches' broom on a branch. Note the callus formation at the base of the branch (arrow).

pulvinus becomes necrotic. By late June the affected foliage is shriveled and may be shed and no additional foliage is produced.

DISCUSSION

M. juglandis appears to be associated with the development of the witches' brooms and galls; it remains to be demonstrated whether or not it is the cause. The leaf tissue from the brooms could be more susceptible than normal leaves to the attacks of this organism.

The extensive overgrowth that occurs would appear to result from more than just the inhibition of the extension of shoots. The presence of a growth stimulus seems likely.

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THE OCCURRENCE AND DISTRIBUTION OF THE CITRUS-ROOT NEMATODE, TYLENCHULUS SEMIPENETRANS COBB, IN FLORIDA[1]

Chancellor I. Hannon[2]

Abstract

The citrus-root nematode, Tylenchulus semipenetrans, occurs wherever citrus is grown in Florida. The nematode is associated with "slow decline" of mature trees as well as young trees replanted in old citrus tree sites. It apparently is more prevalent in the coastal citrus areas of the State than in the interior districts, but its part in decline of coastal citrus plantings is not understood.

Symptoms of slow decline are described and differentiated from those of spreading decline, which is caused by the burrowing nematode, Radopholus similis. Distribution of this citrus-root nematode is chiefly by infected nursery stock. Host range of the nematode and its effect on Florida citrus plantings are discussed.

Although the citrus-root nematode, Tylenchulus semipenetrans Cobb, has been recorded in Florida since 1913 when Cobb (4) identified a few specimens from an unnamed locality, little is known of its distribution in the State. Byars (2) reported finding the nematode in Brooksville, Glen St. Mary, and Gainesville in 1921. Taylor (16) found it associated with all the citrus roots he collected in Orlando in 1944 and he suggested that the nematode was more widely distributed than had been realized.

This paper reports the presently known distribution of the nematode in Florida, describes the symptoms as they are presently understood, lists the known hosts of the nematode, and discusses the potential dangers of replanting infested old grove lands.

In 1947, Suit, DuCharme, and Jones (12) found T. semipenetrans associated with citrus roots from groves affected with spreading decline, a disease at that time of unknown etiology. A year later, Suit, Knorr, and Jones (15) reported finding the nematode in all areas of spreading decline sampled, but only in 9 of 81 healthy groves. In 1949, Suit, Knorr, and DuCharme (13) suggested that the citrus-root nematode might be responsible for spreading decline. However, studies by Suit, Knorr, and Ford (14) in 1950 showed that trees affected with spreading decline but free from citrus-root nematode had symptoms that were different from trees infected with T. semipenetrans alone, indicating that two distinct diseases had been previously considered as one. Of 225 groves examined by DuCharme and Suit in 1954 (6), the citrus-root nematode was found in 47. The nematode was present in areas of spreading decline in only 13 groves and in another 18 groves the nematode was found in areas with decline symptoms that were not those of spreading decline. At present, the nematode is known to occur in citrus groves or nurseries in 35 counties of Florida (7).

Table 1 summarizes survey data compiled by the United States Department of Agriculture, Plant Pest Control Division, and the Division of Plant Industry, Florida Department of Agriculture[3]. The data are based on citrus root samples collected to determine the distribution of Radopholus similis (Cobb) Thorne, the cause of spreading decline disease of citrus. All plant parasitic nematodes found in these samples were recorded so that these records contain considerable information relative to nematodes associated with citrus.

Much attention has been given the citrus-root nematode in California and the "slow decline" disease it causes, and control is recognized as a major prerequisite for the successful replanting of old citrus groves. Moreover, some benefits have been obtained in controlling infestations of the nematode in bearing groves. Little, however, is known about the destructiveness of this nematode on citrus in Florida and no efforts are currently made to control T. semipenetrans prior to replanting old citrus lands. The nematode has been found associated with various citrus declines in Florida and populations of the nematode have conspicuously reduced the growth of infected rough lemon seedlings in greenhouse experiments (8). Poor production in many groves cannot be attributed to any factor common to all trees in the grove except the presence

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[3] Access to these records was granted through the kind cooperation and interest of Mr. James West, Plant Pest Control Division, United States Department of Agriculture and Mr. Charles Poucher, Division of Plant Industry, Florida Department of Agriculture.

Table 1. Distribution of the citrus nematode, Tylenchulus semipenetrans, in commercial citrus groves in Florida based on records of the Florida Division of Plant Industry and the United States Department of Agriculture as of February 1, 1961.

County	Properties		Approximate	Samples	
	inspected	infested	acres infested	collected from infested properties	positive for citrus nematode
Coastal Areas					
Brevard	86	27	757	347	121
Citrus	14	2	18	34	2
Hernando	34	12	661	149	61
Hillsborough	184	77	2,105	1,767	865
Indian River	84	33	1,104	359	161
Lee	16	11	463	75	40
Manatee	41	26	970	270	138
Martin	12	2	51	15	6
Pasco	85	33	1,930	705	267
Pinellas	77	45	2,040	673	309
Sarasota	14	5	218	48	17
St. Lucie	52	21	690	164	66
Volusia	109	73	1,290	658	365
Total	808	367	12,297	5,264	2,418
Interior Areas					
Alachua	15	5	38	42	27
DeSota	65	18	1,177	180	83
Hardee	113	35	1,579	544	294
Hendry	7	5	205	31	15
Highlands	390	23	1,505	1,235	91
Lake	694	38	1,244	1,054	132
Marion	33	4	34	55	24
Orange	596	188	3,412	5,703	416
Osceola	52	14	397	135	60
Putman	31	28	326	233	121
Polk	3,086	481	10,527	33,670	7,765
Seminole	70	31	590	826	389
Sumter	17	3	140	25	7
Total	5,169	873	21,174	43,733	9,424
Misc. Areas					
Charlotte	5	3	215	988	92
Dade	107	5	85	40	17
Palm Beach	10	2	18	12	7
Total	122	10	318	1,040	116
Grand Total	6,099	1,250	33,789	50,037	11,958

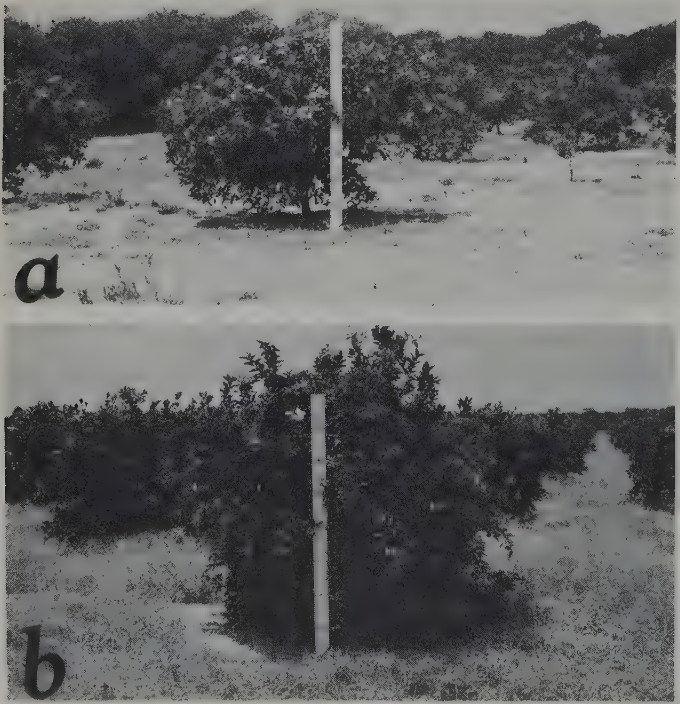

FIGURE 1. Differences between trees planted on soil infested with the citrus-root nematode (A) and trees planted on fumigated soil (B). Trees are Valencia on rough lemon from the same nursery source and planted in the groves in February 1956. The soil type and management program for each grove is similar. Trees in A will yield less than 1 box in 1962 while those in B were picked at a 2.5 box average in March 1962. (Photographs taken in February 1960)

of large populations of T. semipenetrans. Adequate yields in these groves are often obtained only at the expense of increased quantities of fertilizer and water, thus causing a reduction in the net return.

Symptoms of decline associated with infestations of T. semipenetrans of citrus in Florida are very similar to those caused by the same nematode in citrus groves in California. For this reason the name "slow decline," used in California to describe the disease, is being used to describe the disease in Florida.

SYMPTOMATOLOGY

Symptoms of slow decline in aboveground portions of infected trees are not singularly characteristic. In general, the foliage is sparse and often dull gray-green or bronze-green. Leaves are smaller than normal and on severely affected trees they tend to stand upright and to be somewhat cupped. Scaffold branches are readily visible through the poor leaf cover. New flushes of growth are not so numerous or so vigorous as those on healthy trees. Fruit set is reduced and the fruit is often smaller than usual. Many trees showing these symptoms and infected with T. semipenetrans wilt noticeably during the warmest part of the day though soil moisture levels are such that adjacent healthy trees are not in wilt. While there is no record of trees being killed by slow decline in Florida, the disease has rendered many trees worthless. Trees replanted in the site of such trees seldom grow as vigorously as trees replanted in groves where the citrus-root nematode is not a problem (Fig. 1).

In general, symptoms of spreading decline and slow decline are quite different. With spreading decline, the diseased trees are grouped together and are usually associated with an infected reset tree or an infected ornamental suscept growing in the vicinity. Usually few trees exhibit symptoms when the disease is first recognized. However, more trees show symptoms each year so there is an ever enlarging area of diseased trees. Diseased trees are marked by thin, chlorotic foliage, small leaves, twig die-back, absence of older leaves, a heavy bloom in spring and an extremely poor fruit set. Usually there is much less foliage on these trees than on trees affected by slow decline. The symptoms of spreading decline form a well-defined margin or "color break" between the diseased trees and the symptomless, but usually infected, trees on the outer edges of the margin. There is little variation in symptom pattern among trees in the area of decline. On the other hand, the disease caused by T. semipenetrans is characterized by a decline that does not involve a particular group of trees, or does it necessarily affect more trees within the grove each year. Symptoms of decline vary from tree to tree; slow decline may be more noticeable in one tree than in another.

However, once symptoms are evident, the tree seldom again has a vigorous appearance. Lack of any distinct margin between healthy and decline trees is also noticeably absent. Chlorosis of foliage is not a prominent symptom. In some cases trees may support rather large populations of the nematode without evidence of decline symptoms in the aboveground parts.

A crusted appearance not unlike a dock piling overgrown with barnacles is usually characteristic of feeder roots infected with T. semipenetrans. This symptom is produced by particles of sand that adhere to and are imbedded in the mucoid materials secreted by the female nematode into which she lays masses of eggs. Little soil can be removed from such roots with vigorous shaking. Branch roots are rather stubby, brittle, and easily broken. Feeder roots from trees affected with spreading decline, on the other hand, are not encrusted. R. similis is an internal parasite that lays its eggs in the cortical and stelar tissues of the root and does not produce mucoid material. Both nematodes may occur in an area of a grove with decline symptoms but the aboveground symptoms are usually those of spreading decline while roots will usually show symptoms of the citrus-root nematode.

HOST RANGE

In addition to Citrus spp. and related plants, the citrus nematode is also parasitic on olive (species not designated) (1), persimmon (Diospyros virginiana), lilac (Syringa vulgaris), and cultivated grape (Vitis vinifera) in California (9) and in Australia (10). Of these plants, only persimmons and grapes grow in Florida, but the nematode has not been found parasitizing roots of either of these plants. Chitwood and Birchfield (3) reported finding roots of Mikania batatifolia infected by T. semipenetrans near Homestead, Florida and suggested the nematode is endemic. Roots of M. batatifolia examined by the author in the summer of 1960 from the same site failed to yield specimens of the nematode[4]. Larvae somewhat resembling those of T.

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[4] Roots were collected through the cooperation of Mr. W. R. Llewellyn, formerly Assistant County Agent, Dade County, Florida.

semipenetrans were found in root washings of material collected at the same site in 1961. No females or males have been found on *M. batatifolia* either from the Homestead location or from material collected in the vicinity of Lake Alfred. Coconuts (*Cocos nucifera*) near Key West, Florida have also been reported as a suscept (17), but this report could not be confirmed because the trees were destroyed by hurricane Donna in September 1960.

DISCUSSION

That the nematode is distributed chiefly by infested nursery stock rather than because of its reputedly endemic existence is supported by observations in the field and survey data. Cobb (5) first correlated the narrow range suscepts and infected nursery stock with the distribution of *T. semipenetrans*. Groves with uniform infestations often adjoin groves or blocks that are not infested, which suggests the probability that the infestation in one grove or block is due to the source of planting stock. In the past, it has been the practice of many growers to produce nursery stock adjacent to bearing groves or on old grove lands. Available records show that 12% of the citrus nurseries in Florida are infested with *T. semipenetrans*. Stringent regulations are now in effect to prohibit movement of infested nursery stock and to define those sites that may be used for propagation of nursery stock.

Data in Table 1 indicate that the citrus-root nematode is widely distributed in Florida; at least 34, 000 acres of citrus are known to be infested with the nematode. The infested acreage is probably larger because many infested groves have not been included in the surveys conducted by State and Federal agencies. New infested areas are located daily as the spreading decline control work continues. However, this work is confined largely to Polk, Highlands, Orange, and Lake counties of the interior district where spreading decline is most common. Little new information has been collected in the coastal counties since the original burrowing nematode survey was made in 1954-1956.

The distribution of *T. semipenetrans* varies from grove to grove. It has often been found in every sample in some groves; in other groves few samples have been positive. The nematode has been found in about 46% of the samples originating in coastal areas and in only 21% of the samples from the interior districts.

Suit, DuCharme, and Jones (12) first reported the association of *T. semipenetrans* with a decline of citrus (spreading decline) in the interior or Ridge section of Florida. It has been shown since that *R. similis* is responsible for this particular disease and the symptoms of spreading decline are distinct from those of slow decline caused by *T. semipenetrans* (11). Beginning in 1959, a number of groves infested with the citrus-root nematode have been examined for symptoms of slow decline. Groves older than 18 to 20 years with a high population of the nematode usually show decline symptoms, which cannot be attributed to any cause other than the citrus-root nematode. Decline symptoms correlating with large populations of *T. semipenetrans* have been observed on 4- to 5-year-old reset trees in individual replant sites or blocks of replants in established groves. Observations indicate these replants are much less productive than resets planted in soil free of the citrus-root nematode.

While the survey data in Table 1 indicate a more prevalent distribution of the nematode in coastal counties where it occurs in 44% of the properties surveyed, it is difficult to attribute the condition of trees in St. Lucie, Indian River, and Brevard counties to the presence of citrus-root nematode alone. Most groves in this area are planted in rather shallow, poorly drained soils. Trees are seldom rooted deeper than 18 inches and are frequently subjected to flood conditions during the rainy seasons. Populations of citrus-root nematode in these declining groves are seldom as large as populations in declining groves in the interior regions where citrus is generally grown on deep sands.

The decline in East Coast groves seems more complex; it may well be due to poor root growth in a shallow, poorly drained soil compounded by the presence of citrus-root nematode.

Thousands of acres of citrus groves in Florida were planted after the disastrous freeze of 1894-1895 and prior to World War I. Many of these groves for one reason or another have become unproductive. Surveys have shown many of these groves to be infested with *T. semipenetrans*. As this acreage is replanted, growers will become more aware of the importance of the citrus-root nematode when reset trees do not make the normal thrifty growth expected of them. This, more than any other manifestation of the nematode's destructiveness, will call the growers' attention to the importance of the problem.

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CITRUS EXPERIMENT STATION, LAKE ALFRED, FLORIDA

AN AGITATOR-INCUBATOR FOR ASEPTIC CULTURING OF VIRUS-INFECTED CELLS AND TISSUE IN LIQUID MEDIA

H. H. Thornberry[1]

INTRODUCTION

In a study of viral infections in aseptic culture of plant cells and tissues, liquid media was used instead of semi-solid media for certain problems being investigated. This avoids the use of gelling agents, such as agar and Noble agar, that might introduce unknown or uncontrollable variables in the culture media. Since available apparatus for aerating cultures in liquid media were not particularly suitable for the studies on viral infected tissues, an agitator encased within a dust-proof transparent incubator was designed and constructed. Only commercially available equipment and materials were used. This paper describes the agitator-incubator and provides instructions for its construction.

DESCRIPTION OF APPARATUS[2]

The agitator-incubator consists of four basic parts: a portable base (small desk), incubator, agitator, and lighting system (Figs. 1 and 2).

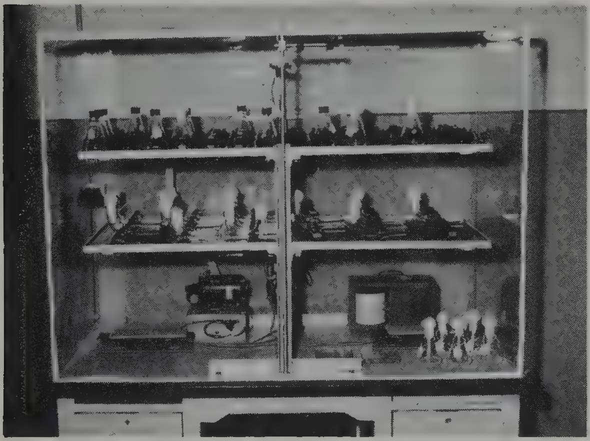

FIGURE 1. Photograph of agitator-incubator with door removed for better vision. Lower part of base (desk) excluded from the picture. Light system not attached.

The portable base is a small wooden desk (25 x 50 x 29 inches) mounted on four casters under the outside corners of the desk. A four-receptacle electrical box mounted at a convenient place on the vertical surface provides for all demands of electric current. The electrical box has a single extension cord with a 90 degree male plug for a 115 volt wall receptacle. A 5/16 inch sheet of transite serves as a fireproof cover for the desk top and as a smooth floor for the incubator.

The incubator is a transparent case (1/4-inch Plexiglass) with heating elements and controls. The dimensions of the case are 3 feet 1/4 inch high x 4 feet wide x 2 feet 1/4 inch deep (outside dimensions). The case consists of a 3 x 4 foot sheet for the back, two 2 x 3 foot sheets for the ends, and a 2 x 4 foot sheet for the top. The Plexiglass is cemented with a thick paste of Plexiglass dissolved in ethylene dichloride. (Acetone or a mixture of acetone and ethylene dichloride may be used to dissolve Plexiglass). The front (a removable door) of the case consists of a 3/16-inch 3 x 4 foot sheet of Plexiglass held in place by ten spring hooks at the sides and top and by an adjustable angle-iron base bar with two bolts and winged nuts. The front edge of the top sheet of Plexiglass is reinforced with a bar of angle iron which is supported at the middle by a vertical Castaloy rod anchored on the metal base support of the agitator. The door facings are stripped with foam rubber which provides a dustproof seal when the door is in place and tightened. The heating elements are two 150-watt strip heaters controlled by a mercury thermoregulator set at 26° C and a thermostat relay. A signal light is connected to the heating

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[1] Professor of Plant Pathology, Department of Plant Pathology, University of Illinois, Urbana.

[2] Detailed specifications for the construction of this apparatus may be obtained from the author upon request.

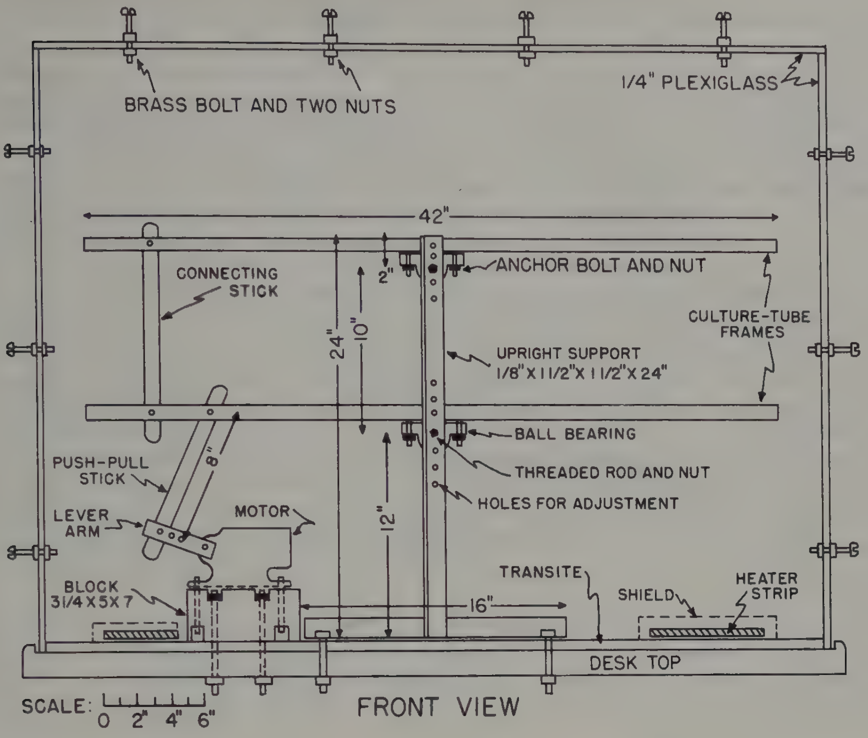
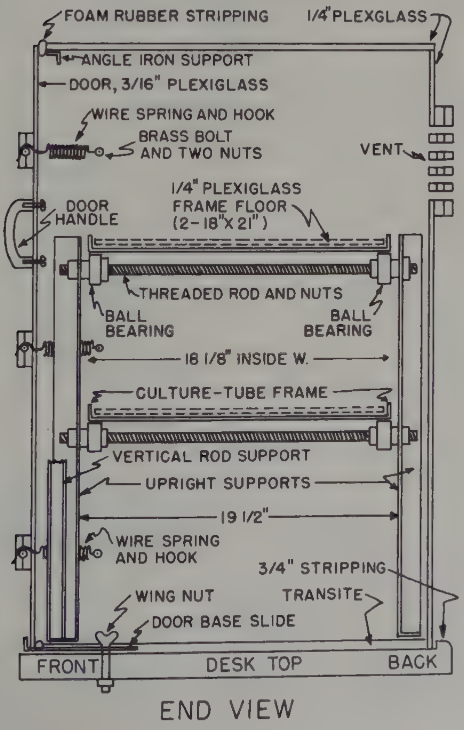

BRASS BOLT AND TWO NUTS
1/4" PLEXIGLASS
42"
2"
24"
10"
ANCHOR BOLT AND NUT
CONNECTING STICK
CULTURE-TUBE FRAMES
UPRIGHT SUPPORT 1/8"X 1 1/2"X 1 1/2"X 24"
BALL BEARING
PUSH-PULL STICK
8"
THREADED ROD AND NUT
HOLES FOR ADJUSTMENT
12"
LEVER ARM
MOTOR
TRANSITE
BLOCK 3 1/4 X 5 X 7
16"
SHIELD
HEATER STRIP
DESK TOP
SCALE: 0 2" 4" 6"
FRONT VIEW

FIGURE 2. Drawing of agitator-incubator showing individual parts in a front view and an end view.


FOAM RUBBER STRIPPING
1/4" PLEXGLASS
ANGLE IRON SUPPORT
DOOR, 3/16" PLEXIGLASS
WIRE SPRING AND HOOK
BRASS BOLT AND TWO NUTS
VENT
1/4" PLEXIGLASS FRAME FLOOR (2-18"X 21")
DOOR HANDLE
THREADED ROD AND NUTS
BALL BEARING
BALL BEARING
18 1/8" INSIDE W.
CULTURE-TUBE FRAME
VERTICAL ROD SUPPORT
UPRIGHT SUPPORTS
19 1/2"
WIRE SPRING AND HOOK
3/4" STRIPPING
WING NUT
TRANSITE
DOOR BASE SLIDE
FRONT
DESK TOP
BACK
END VIEW

elements. Dustproof ventilation is provided by a multiple-hole-slide plate (plugged with cotton) on the back side near the top and by two 1 1/2 inch holes (plugged with cotton) in the floor of the incubator. These holes are fitted with plumbing pipe and fittings so that gas tubing may be attached for introducing air, gases, or gas mixtures into the incubator under a slight positive pressure. The upper ventilator may be closed by removing the cotton plugs and replacing them with cork or rubber stoppers or by moving the Plexiglass slide plate to close the holes. Temperature within the incubator varies less than ± 0.5°.

The agitator is a suspended seesaw which is propelled by a slow-speed electric direct current motor. The speed of the motor is regulated by a current controller which transforms accessible 115 volt A. C. into 115 volt D. C. Direct current is preferable for adjustable control of motor speed and in turn the rate of agitation (number of times the culture tubes tilt per minute). The current controller is mounted outside the incubator (in a desk drawer) for accessibility of adjustment controls and dissipation of emitted heat without influencing the temperature inside the incubator. The tilt of the culture-tube frame is adjustable by attaching the push-pull stick (a connecting stick between the culture-tube frame and the motor lever) to another hole in the lever arm strapped to the drive shaft of the motor. The turning of the motor and lever arm propels the seesaw movement of the culture-tube frames. A connecting stick between the upper and lower culture-tube frames tilts the two frames simultaneously. The floor of the culture-tube frames are 1/4-inch Plexiglass sheets with 1/4-inch holes spaced 1 inch apart. The holes provide gentle air circulation and a place for attaching spring-clamp holders of culture tubes. The transparent Plexiglass floors of the culture-tube frames permit the illumination of liquid medium cultures on the lower frame and also stationary cultures in various containers on the floor of the incubator.

The lighting system consists of a 48-inch fluorescent fixture (three 40-watt tubes) mounted 8 inches above the incubator. The quantity of light is controlled by removing one or two of the tubes. A black plastic cover for the incubator may be used to exclude all light.

The apparatus illustrated in Figure 1 was constructed at a cost of about $500.00 for materials and about 30 man-hours of labor.

Acknowledgment: The author is grateful to W. M. Bever for his critical review of the manuscript, to Messrs. C. J. Rund and R. F. Shields for their helpful suggestions and technical skill in constructing a major portion of the apparatus, and to Bodine Electric Motor Company for recommending a satisfactory motor and current controller.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS

A MICROVOLUMETER FOR MEASURING THE VOLUME OF PLANT TISSUE CULTURES

H. H. Thornberry and Inez N. Kamp[1]

In studies on virus-infected and normal plant tissues in aseptic culture, it was desired to measure the volume of cultures without destroying the cultures for other uses. To obtain these measurements we designed and assembled a simple apparatus for measuring volumes from 0.01 to 10 ml. This paper describes the apparatus, depicts the operations required for measurements, and reports findings on some measurements.

DESCRIPTION OF APPARATUS

The apparatus, designed on the principle of liquid displacement by objects submerged into the measuring fluid, consists of the following basic parts (Fig. 1):

1. Support, a metal rod and stand with clamps.
2. A glass reservoir tube with angled side arm tube (prepared by glass blower, A. F. Robbins).
3. A microburette for collecting the liquid displaced by the tissue cultures and cage. (Tip of burette removed to facilitate the flow of liquid and to avoid clogging).
4. Tissue cage for submerging the cultures in the reservoir fluid.

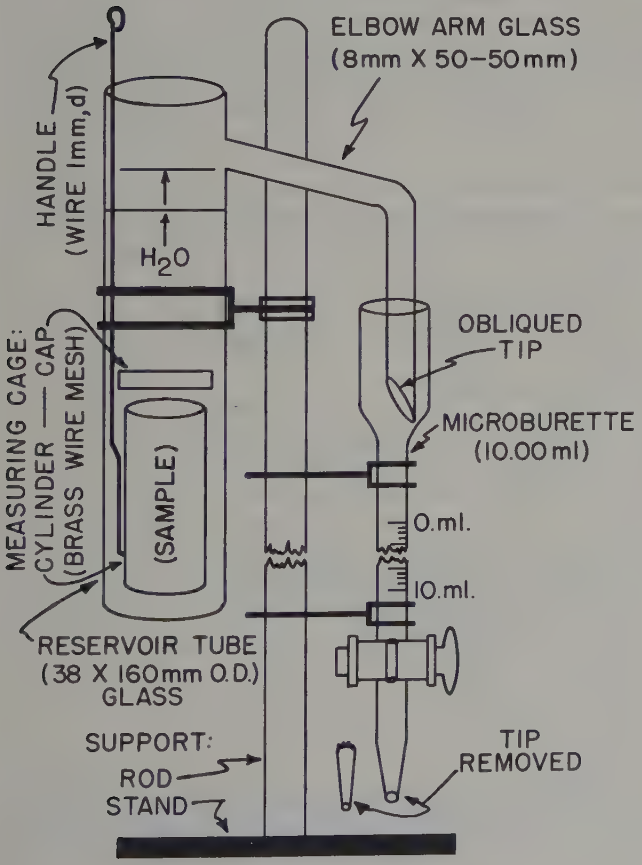

HANDLE (WIRE 1mm,d)
ELBOW ARM GLASS (8mm X 50-50mm)
H_2O
OBLIQUED TIP
MICROBURETTE (10.00 ml)
0.ml.
10.ml.
MEASURING CAGE: CYLINDER — CAP (BRASS WIRE MESH)
(SAMPLE)
RESERVOIR TUBE (38 X 160mm O.D.) GLASS
SUPPORT: ROD STAND
TIP REMOVED

FIGURE 1. Picture of assembled apparatus.

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[1] Professor of Plant Pathology and Research Associate, respectively, Department of Plant Pathology, University of Illinois, Urbana, Illinois.

OPERATIONS

Measurements involve the following operations:

1. Assemble the apparatus (Fig. 1).
2. Add displacement fluid (25% aqueous ethanol) to the reservoir tube until enough liquid flows through the side arm to fill the microburette to the region of graduations.
3. Lower the meniscus of the fluid in the burette to the lowest graduation, 10 ml.
4. Submerge the empty cage into the reservoir fluid to determine the volume of liquid displaced by the cage. This standardization (nos. 2, 3, and 4) should be repeated after each assembly of apparatus.
5. Read the graduation at the meniscus. (This graduation subtracted from 10 ml equals the volume of the cage--inverted graduations would be additive).
6. Lift the basket from the reservoir fluid slowly.
7. Place specimen in cage and repeat operations no. 2 through 6. (cage with specimen in operation no. 4 to determine the volume of the cage plus the specimen--volume of cage with specimen minus volume of empty cage equals the volume of the specimen).

MEASUREMENTS

Water used as a displacement fluid did not flow freely in the apparatus, presumably because of its high surface tension. Some aqueous solutions of substances with low surface tension were tested. Saponin even at 0.01% solution tended to foam, and nonanic acid was too insoluble in water for a satisfactory medium. Aqueous ethanol was satisfactory. The data in Table 1 indicate that less variability of measurements occurred with 50% ethanol than with 25% ethanol. A 25% solution of ethanol in distilled water, however, is recommended as a displacement medium to reduce the amount of tissue shrinkage that might occur with higher concentrations of ethanol.

Table 1. Variability of measurements on the volume of the empty cage with aqueous ethanol as displacement fluid.

Aqueous ethanol	No. of tests	Mean (ml)	Standard deviation	Coefficient of variability (%)
50%	10	2.57	±0.196	7.6
25%	10	2.37	±0.240	10.1

Table 2. Duration of time necessary for equilibration of apparatus with 25% aqueous ethanol as displacement liquid.

Time (minutes)	No. of tests	Mean (ml)	Standard deviation	Coefficient of variability (%)
1	10	2.10	±0.167	7.9
2	10	2.28	±0.057	2.5
3	10	2.25	±0.053	2.3
5	10	2.05	±0.057	2.7

Some measurements on the volume of the empty cage were made to determine the variability of findings on the time allowed for the displacement liquid (25% aqueous ethanol) to collect in the burette. According to the data in Table 2, one minute is insufficient for equilibration. Reasonable degree of equilibration occurred in 2, 3, or 5 minutes. A 2-minute equilibration may be used to conserve time.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS

TOBACCO FROGEYE CONTROL IN VENEZUELA BY ISOLATION OF PLANT BEDS FROM NATIVE VEGETATION[1]

W. D. Valleau[2]

Tobacco, both burley and flue-cured, is grown in Venezuela in very large plantings. It is set near the end or after the end of the rainy period and is irrigated by gravity. During the plant bed season the native vegetation is preparing for the dry season which lasts 5 or 6 months.

The frogeye fungus, Cercospora apii Fres. (2), more commonly called C. nicotianae Ell. & Ev., has been shown by Johnson and Valleau (2) to have a very wide host range - at least 11 families - so that it may be expected to be found on many species of the native vegetation in Venezuela. With the advent of the dry season, when much of the vegetation dies or becomes dormant, one would expect that sporulation of Cercospora would cease.

In Kentucky infection in plant beds is relatively rare, although an occasional spot is sometimes found at pulling time. In Venezuela and in Central America, where plant beds are commonly placed at the edge of the field next to native vegetation and watered daily, frogeye is common in plant beds, especially at second pulling. When infected plants are set in the field, each can act as a center of infection for surrounding plants. With irrigation, and in some areas dews, conditions are favorable for spread and the disease sometimes reduces quality of the lower leaves considerably.

If plant bed infection could be prevented completely and only clean plants set in the very large fields during the dry season when inoculum is scarce, an outbreak could not be expected to occur. While a barn of first primings of flue-cured tobacco was being examined, leaves were noticed that were completely free from frogeye and other leafspots and would grade high as compared with the usual heavily spotted crops. In seeking a reason for this, we found that the plant beds for two seasons had been placed in the middle of a large plowed area well away from native vegetation, and in neither year was there any appreciable frogeye in the harvested crop. This led to the observation that wherever beds were partially surrounded by native vegetation infection in the field was general, but where beds were placed well away from native vegetation in large cultivated areas, the tobacco crop when cured was free from spotting.

This observation not only opens the way to eliminating losses from frogeye in areas where large acreages are planted, but also gives added evidence that the Cercospora that attacks tobacco is not one specific for tobacco, as Chupp (1) implies, but rather is one with a wide host range among native plants. To what extent this method of control can be used in the smaller acreages in the United States is not known, but it should be of some value in larger plantings.

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2. JOHNSON, E. M., and W. D. VALLEAU. 1949. Synonymy in some common species of Cercospora. Phytopathology 39: 763-770.

KENTUCKY AGRICULTURAL EXPERIMENT STATION, LEXINGTON

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

[2] Professor of Plant Pathology (Emeritus).

REDUCTION IN YIELD OF COTTON CAUSED BY DISEASES IN 1961

Compiled by the Cotton Disease Council, Committee on Disease Losses: Harlan E. Smith, Chairman; A. L. Smith, W. E. Cooper

The accompanying tabulated summary of the 1961 Cotton Disease Loss Estimates is the tenth report submitted by the committee on cotton disease losses. The summary has been compiled from 49 State estimates. The accuracy of the estimates is highly reliable because most of the same cooperators have contributed for the last 10 years and, as a consequence, their methods and techniques are becoming uniform. The committee feels that considerable credence can be placed in the report.

Table 1. ESTIMATED REDUCTION IN 1961 COTTON YIELD AS A RESULT OF DISEASE DAMAGE

Disease	Calif.	Ariz.	N. Mex.	Tex.	Okla.	Mo.	Ark.	La.	Miss.	Ala.	Ga.	S. C.	N. C.	Tenn.	Bales lost	% loss
1. FUSARIUM WILT *Fusarium vasinfectum*	--	--	--	0.25	0.60	3.00	1.25	5.00	1.00	2.00	4.25	1.90	1.50	0.05	161,907	0.94
2. VERTICILLIUM WILT *Verticillium albo-atrum*	2.00	3.50	8.00	5.51	2.33	4.00	3.40	1.00	2.25	tr	--	--	0.40	2.66	584,129	3.40
3. BACTERIAL BLIGHT *Xanthomonas malvacearum*	tr	tr	0.20	4.33	3.16	2.50	0.20	0.50	0.50	0.30	2.50	0.55	0.50	0.45	329,931	1.92
4. ROOT ROT *Phymatotrichum omnivorum*	tr	2.32	tr	5.65	0.13	--	tr	tr	--	--	--	--	--	--	364,617	2.12
5. ANTHRACNOSE BOLL ROT *Glomerella gossypii*	--	--	--	0.10	0.25	tr	tr	tr	0.75	1.00	1.25	0.90	0.20	0.50	44,821	0.26
6. SEEDLING DISEASES *Rhizoctonia*, etc.	1.50	2.78	2.15	2.51	3.16	2.50	4.75	5.00	2.50	4.00	4.50	3.25	5.00	9.66	551,404	3.20
7. ASCOCHYTA BLIGHT *Ascochyta gossypii*	--	--	--	0.29	--	1.00	0.10	tr	0.25	6.00	1.05	0.70	0.20	0.22	88,095	0.51
8. BOLL ROTS *Rhizopus*, etc.	4.00	0.83	tr	0.42	2.83	0.10	0.40	10.00	2.00	3.00	2.25	4.45	1.00	0.41	295,311	1.71
9. ROOT KNOT *Meloidogyne* sp.	3.00	1.82	1.50	1.03	0.33	3.00	tr	4.00	0.50	4.00	3.00	5.00	5.00	0.50	288,374	1.67
10. OTHERS	0.75[a]	0.55[b]	tr	1.53[c]	--	10.00[d]	2.10[e]	1.00	--	--	0.25	0.05	0.10	0.08	206,909	1.20
TOTAL PERCENT LOSS	11.25	11.80	11.85	21.62	12.79	26.10	12.20	26.50	9.75	20.30	19.05	16.80	13.90	14.53		16.93
TOTAL BALES LOST IN HUNDREDS	2,151	1,103	402	13,101	526	1,318	2,021	1,730	1,765	1,591	1,214	833	451	942	29,154[g]	
YIELD IN THOUSAND BALES 1961[f]	1700	825	300	4750	360	375	1455	480	1635	625	515	413	280	555		

[a] Leaf crumple virus and unknown leaf spot [b] Rust (*P. cacabata*) and crazy top [c] Rust (*P. cacabata*) and others [d] Alternaria and Cercospora leaf spots
[e] Minor leaf spots and others [f] Dec. 8, 1961 USDA, AMS Estimate [g] In hundreds

COTTON DISEASE COUNCIL, COMMITTEE ON DISEASE LOSSES

THE OCCURRENCE OF DUTCH ELM DISEASE IN OKLAHOMA

John E. Thomas[1] and Clyde A. Bower[2]

Since 1959 the Dutch elm disease has been known to be present in Cherokee and Montgomery counties, Kansas[3], adjacent to the Oklahoma border. During the summer of 1961 suspected diseased trees were found in Miami, Ottawa County, Oklahoma. Positive identification of Ceratocystis ulmi (Buism.) C. Moreau was made by the U. S. Forest Service Pathology Laboratory at Columbia, Missouri. This is the first authenticated report of this disease in Oklahoma. Other suspected trees have been observed in Nowata County, but positive identification is still lacking.

OKLAHOMA STATE UNIVERSITY, STILLWATER AND OKLAHOMA STATE BOARD OF AGRICULTURE, OKLAHOMA CITY

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[1] Department of Botany and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma.

[2] Oklahoma State Board of Agriculture, Capitol Building, Oklahoma City, Oklahoma.

[3] Kramer, C. L., and Hugh E. Thompson. 1960. Dutch elm disease in Kansas in 1959. Plant Disease Reptr. 44: 162.

THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

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Volume 46 Number 7

July 15, 1962

3 AUG 1962

Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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(3) ABSTRACTS are requested for all except very short articles.

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so.

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The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to twelve double-spaced typed pages, including tables, graphs, and photographs. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases.

Paul R. Miller

Manuscripts for and correspondence about this publication
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Plant Industry Station, Beltsville, Maryland

THE PLANT DISEASE REPORTER

Crops Research Division
Volume 46

Plant Industry Station, Beltsville, Maryland
Number 7

July 15, 1962

CONTENTS

1. The effect of plant growth regulators on five genera of bacteria
LINNEA STEWART 469

2. Effect of some single element deficiencies on pathogenicity of Pratylenchus penetrans to pepper plants
M. FAROUK SHAFIEE and W. R. JENKINS 472

3. Effects of certain mixtures of fungicides and spray additives on the control of peach scab and brown rot
W. A. CHANDLER 476

4. A new wilt disease of maize in Egypt
A. S. SAMRA, et al. 481

5. Some observations on the number of root lesion nematodes necessary to cause injury to seedling onions
JOHN M. FERRIS 484

6. Target blotch of sugarcane in Florida
EDWIN H. TODD 486

7. Temperature factors in the control of black spot of citrus
ROY J. SMITH 487

8. Calcium nitrate for field control of tomato southern blight in South Carolina
W. R. SITTERLY 492

9. Peach mosaic virus in horticultural plum varieties
T. S. PINE and L. C. COCHRAN 495

10. Stem and crown necrosis of Coastal Bermuda grass caused by Helminthosporium spiciferum
ROBERT T. GUDAUSKAS 498

11. Root knot and the seedling disease complex of cotton
L. V. WHITE 501

12. Transmission of some gladiolus viruses on tools in harvesting flowers and corms
PHILIP BRIERLEY 505

13. Phytophthora crown rot of petunia
DOUGLAS J. PHILLIPS and RALPH BAKER 506

14. Effectiveness of Dyrene in controlling Stemphylium leaf spot of birdsfoot trefoil
CHARLES R. DRAKE 509

15. Spray programs and the control of sooty blotch of apples
ROBERT H. DAINES 513

16. Experiments with DBCP in established vineyards
D. J. RASKI 516

17. Fomes annosus on shortleaf pine in Missouri
F. H. BERRY and O. J. DOOLING 521

18. Interrelationships of bud-perpetuated conditions in prune trees, and variations in these conditions with time
A. W. HELTON 523

19. Stability of crinkle symptoms in prune trees
A. W. HELTON 528

20. Susceptibility of peach rootstocks to root-knot nematodes
G. MINZ and E. COHN 531

21. A report on the testing of onion varieties for resistance to lesion nematodes
G. B. BERGESON 535

22. Reaction of beans to tomato and tobacco strains of tobacco mosaic virus
E. M. JOHNSON 537

23. Pythium damping off in tobacco plant beds
E. M. JOHNSON 539

24. Sterilization of wheat seed by ethylene oxide
JAMES F. SCHOEN 540

25. First record of hop cyst nematode (Heterodera humuli Filipjev, 1934) in the United States
GRACE S. COBB 541

THE EFFECT OF PLANT GROWTH REGULATORS ON FIVE GENERA OF BACTERIA[1]

Linnea Stewart

Summary

Growth of Erwinia amylovora, Pseudomonas caryophylli, Xanthomonas pruni, Bacillus subtilis, and Escherichia coli was retarded by phosfon at concentrations from 10^{-2}M to 10^{-5}M. Growth of E. coli was retarded by carvadan, a quaternary ammonium growth retardant, and by four quaternary ammonium germicides.

INTRODUCTION

The plant regulating activity of chemicals has been evaluated in may ways. Retardation of the abscission of mature fruits of apples was used by Marth, et al. (2). Others have used cell elongation or multiplication, formative growth effects, parthenocarpy, modification of organs, repression or stimulation of root or stem growing points, and many others (3). Bacteria sensitive to plant growth regulators would be efficient organisms for screening for such biological activity.

MATERIALS AND METHODS

Three laboratory strains of Escherichia coli Migula and one each of Bacillus subtilis Cohn, Erwinia amylovora (Burr.) Winslow et al., Pseudomonas caryophylli Starr & Burkh., and Xanthomonas pruni (E. F. Sm.) Dows. were grown in Difco nutrient broth cultures. The following chemicals were tested for their effect on the growth of these bacteria: two plant growth-retardants, phosfon (2,4-dichlorobenzyltributylphosphonium chloride) and carvadan (3-isopropyl-4-dimethylamino-6-methylphenyl-1-piperidinecarboxylatemethyl chloride), an herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid), salt (sodium chloride), and four quaternary ammonium germicides also active against fungi and algae, Hyamine 1622 (di-isobutylphenoxyethoxyethyldimethylbenzylammonium chloride), Hyamine 10X (di-isobutylcresoxyethoxyethyldimethylbenzylammonium chloride), Hyamine 3500 (alkyldimethylbenzylammonium chloride), and Hyamine 2389 (40% methyldodecylbenzyltrimethylammonium chloride, 10% methyldodecylxylylenebistrimethylammonium chloride, 50% water).

The chemicals were dissolved in warm water at twice the highest molar concentration used and passed through a Seitz bacteriological filter. The stock solutions were serially diluted and 5 ml aseptically pipetted into tubes with 5 ml of autoclaved double-strength nutrient broth. For control tubes 5 ml of sterile distilled water were added to tubes with 5 ml of double strength broth. Experimental tubes were inoculated by transfer of a 3-mm loop of bacteria from a 48-hour culture grown at 84° F. All tubes were incubated for 24 hours at 84°, except X. pruni which was incubated for 72 hours, and growth estimated by measurement of turbidity in a Klett-Summerson colorimeter adjusted to zero reading with an uninoculated control tube of nutrient broth. Differences in turbidity between control and treated tubes inoculated with E. coli could first be seen in about 6 hours. The other bacteria grew more slowly but the control tubes of all except X. pruni were very turbid after 24 hours' incubation. The number of bacteria in representative sample cultures of E. coli was determined by dilution to extinction and dilution plate counts on nutrient agar in Petri dishes. A curve constructed by plotting the colorimeter readings for serial dilutions of cultures of E. coli of a known concentration against the number of bacterial cells showed the expected logarithmic relationship.

For purposes of comparison between different tests, the growth of the bacteria in treatments is presented as percentage of growth in the control tubes. The viability of the bacteria in several treatments where growth has been inhibited was tested by further incubation and by transfer to standard nutrient broth.

In each test the treatments were replicated four times and the readings subjected to analysis of variance. The significance of the differences between treatment means was determined by Duncan's multiple range test.

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[1] This paper represents the results of a high school science fair project which received distinction at both the local science fair and the National Science Fair - International in 1962.

RESULTS

Phosfon, carvadan, and the Hyamines significantly inhibited the growth of E. coli at the higher molar concentrations used (Table 1). The Hyamines were inhibitory at lower molar concentrations than phosfon or carvadan and stimulated growth at 10^{-6} molar concentration. Salt and 2,4-D did not affect the growth of E. coli at the concentrations tested.

The effect of different molar concentrations of phosfon on the growth of several genera of bacteria is shown in Table 2. Growth of all the bacteria was retarded by the higher concentrations of phosfon. The several strains of E. coli and the other genera varied in their response to the lower concentrations of phosfon. Dilute solutions did not stimulate growth.

Table 1. The effect of five concentrations of phosfon, 4 related Hyamine compounds, carvadan, 2,4-D, and salt upon growth of *Escherichia coli* expressed as percentage of growth of control cultures.

Code name	Molecular weight	Molar concentration					
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Control
Phosfon	362	9a[a]	6a	75b	108c	96c	100c
Hyamine 1622	466	--[b]	--	16a	10a	122c	100b
Hyamine 10X	480	--	--	28b	2a	131d	100c
Hyamine 3500	358	--	--	28b	4a	134d	100c
Hyamine 2389	331	--	--	13a	103b	118c	100b
Carvadan	280	35a	53b	91cd	81c	88c	100d
2,4-D	220	--	102a	97a	107a	101a	100a
Salt	56	104a	97a	107a	109a	111a	100a

[a] For any chemical, values not followed by the same letter are significantly different at the 5% level.

[b] Values are missing for concentrations at which chemicals were not soluble.

Table 2. The effect of five concentrations of phosfon on the growth of three strains of *Escherichia coli*, and one strain of *Bacillus subtilis*, *Erwinia amylovora*, *Pseudomonas caryophylli* and *Xanthomonas pruni*, expressed as percentage of growth of control cultures.

Organism tested	Molar concentration					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Control
Escherichia coli strain 1	9a[a]	6a	75b	108c	96c	100c
Escherichia coli strain 2	2a	70b	111bc	126c	133c	100bc
Escherichia coli strain 3	2a	2a	55b	83c	99c	100c
Bacillus subtilis	12a	12a	63b	85c	95c	100c
Erwinia amylovora	5a	27b	79c	89d	87d	100e
Pseudomonas caryophylli	4a	60b	97c	97c	97c	100c
Xanthomonas pruni	2a	10ab	18b	82c	99d	100d

[a] For any bacteria, values not followed by the same letter are significantly different at the 5% level.

Tubes with phosfon at 10^{-2} molar concentration inoculated with E. coli, E. amylovora, or P. caryophylli were clear after 24 hours' incubation at 84° F. After 96 more hours at 84° the tubes were still clear. Transfers to standard nutrient broth after the first 24 hours of incubation showed no subsequent growth. In tubes with phosfon at 10^{-3} these three bacteria were strongly inhibited during the first incubation for 24 hours at 84°. However, these tubes and control tubes inoculated from them were very cloudy after 96 hours at 84°.

All the Hyamines at a concentration of 10^{-4} M completely inhibited E. coli. At 10^{-5} Hyamine 1622, 10X, and 3500 showed inhibition for only 24 hours. The mixtures of Hyamines at 2×10^{-2}M and 2×10^{-3} molar concentration with double strength nutrient broth immediately became very turbid and in this respect resembled tubes with heavy bacterial growth. The mixture with 10^{-4}M resulted in some slight immediate turbidity but none was evident at lower concentrations. Carvadan at 10^{-2} inhibited E. coli for only 24 hours.

DISCUSSION

Plant growth retardants and the quaternary ammonium germicides produced similar reductions in the growth of E. coli. The germicides were effective at lower molar concentrations but the growth retardants repressed growth over a greater range of concentrations. Both were bactericidal at the higher concentrations and bacteriostatic at intermediate dilutions. The stimulation of growth of E. coli at a concentration of 10^{-6}M of the Hyamines was not found with carvadan or phosfon. However, unpublished results with structural variants of phosfon showed similar stimulation of growth at low concentrations.

The sensitivity of E. coli to both carvadan and phosfon suggests the use of this organism to screen chemicals as potential plant growth retardants or for other biological activity. Within a few hours a large range of concentrations of a chemical can be tested on large numbers of individual organisms under easily controlled conditions. In higher plants translocation of the chemical from the point of application to the tissue where it is active makes it difficult to determine the actual effective concentrations. The direct exposure of single-celled organisms seems to eliminate many of the problems involved in determining the mode of action of growth regulators in the tissue of higher plants.

Phosfon retarded the growth of all five widely different genera of bacteria tested. Three of the bacteria were plant pathogens and the interaction of the pathogens and the growth retardant in the host plants would be of interest. The wide range in sensitivity within E. coli and also between the different plant pathogens is comparable to the results reported on higher plants (1). The right combination of tolerance of the host plant and sensitivity of a pathogen to a growth regulator might make some degree of disease control possible.

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EFFECT OF SOME SINGLE ELEMENT DEFICIENCIES ON PATHOGENICITY OF PRATYLENCHUS PENETRANS TO PEPPER PLANTS[1]

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Abstract

Pepper plants were grown in sand culture and provided with five different nutrient solutions: complete solution, and solutions deficient for N, P, K, and Ca. Except for P-deficient plants, growth of all inoculated plants was, in comparison with uninoculated checks, retarded as a result of nematode infection. Top weight varied according to these various treatments, but was most markedly reduced in plants deficient in nitrogen.

Diseased roots of K-deficient plants were low in phosphorus, which nonetheless accumulated in their leaves, while those of P-deficient plants were low in K.

INTRODUCTION

Nematode infection may result in either an increase or a decrease in concentration of one or more of the elements required for normal growth by the plants. The effect of certain plant-parasitic nematodes on the chemical composition of their hosts has been demonstrated by several investigators, but there is contradiction in the results reported, probably because these investigators were dealing with different hosts; the chemical nature of the soil is also an important factor. When Bessey (1) was conducting several fertilizer experiments with various plants growing in soil heavily infested with root-knot nematodes, he found that potassium exerted a "remarkable beneficial effect upon the plants" and that they yielded a good crop despite the presence of nematodes. He also found that phosphate fertilization did not improve plant growth. In an attempt to control "potato sickness," Ellenby (3) applied solutions of various trace elements to potato plants growing in soils naturally infested with Heterodera rostochiensis, and found that boric acid, zinc sulfate, and manganese chloride applications resulted in increased yield. Ellenby concluded that "potato sickness" would be more severe in soil deficient in boron, zinc, and manganese. Magistad and Oliveira (6) reported that applications of nitrogen markedly increased the percentage of nitrogen in both infected and non-infected plants, but that these applications resulted in increased growth only in those not infected. Van Gundy and Martin (8) investigated the influence of Tylenchulus semipenetrans on the growth and chemical composition of sweet orange seedlings in soils of various exchangeable-cation ratios. They found that leaf copper was reduced by nematodes, particularly where the soils were high in calcium carbonate, sodium or potassium. Leaf sodium was higher in nematode-infected plants than in uninfected plants grown in soils high in sodium.

The objectives of the present experiment were: (a) to study the effect of various nutrient solutions on pathogenicity of Pratylenchus penetrans to pepper, and (b) to determine whether nematode influence on the chemical composition of pepper plants varies with different nutrient solutions provided to the plants.

MATERIALS AND METHODS

Glazed, 3-gallon percolators were washed thoroughly and a piece of glass wool placed over the drain hole of each. They were filled to within 2 inches of the top with washed, dry, quartz sand, and plastic tubes were inserted in their drain holes through rubber stoppers. For each percolator containing sand, a 1-gallon crock was cleaned and placed on the ground to serve as a reservoir for the drained nutrient solution. Each reservoir was covered with plastic to avoid contamination.

The sand was pre-washed with tap water for 5 to 10 minutes, rinsed with 1.0 N HCl for 15 minutes and with 1.0 N NaOH to neutralize the acid. After drainage of the prewash, percolators were flushed with distilled water.

Pepper seeds were germinated directly in the sand of the culture vessels. When seedlings reached suitable size they were thinned to three plants per percolator, and when 4 weeks old they were inoculated with a sterile axenic suspension of Pratylenchus penetrans. One thousand nematodes were placed around the base of the stem of each plant. To avoid washing the

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers, the State University.

Table 1. Composition of the nutrient solutions used to determine the effect of some single element deficiencies on pathogenicity of *Pratylenchus penetrans* to pepper.

Nutrients mg/liter	Treatment: Complete[a]	Less K	Less N	Less P	Less Ca
N	140	140	14	140	140
P	31	31	31	3	31
K	195	16	195	195	195
Ca	200	200	200	200	20
Mg	49	49	49	49	49
Mn	.25	.25	.25	.25	.25
Zn	.05	.05	.05	.05	.05
Cu	.01	.01	.01	.01	.01
Fe	.5	.5	.5	.5	.5
B	.1	.1	.1	.1	.1
S	129	71	129	129	129
Mo	.01	.01	.01	.01	.01

[a] After Robbins (7).

Table 2. Mean fresh weight of nine pepper plants inoculated with *Pratylenchus penetrans* under five different treatments compared with uninoculated plants.

	Fresh weight (in grams)			Growth retardation (%)	
Treatment	tops	roots	total	tops	roots
Complete					
Control	49.3	38.0	87.3		
Infected	45.2	27.5	72.7	8	28
K deficient					
Control	12.0	9.0	21.0		
Infected	9.6	8.0	17.6	20	11
N deficient					
Control	7.2	4.6	11.8		
Infected	2.6	2.0	4.6	64	56
P deficient					
Control	1.1	0.8	1.9		
Infected	1.6	1.9	3.5	-	-
Ca deficient					
Control	48.0	33.1	81.1		
Infected	40.5	32.0	72.5	16	4

nematodes from around the roots, plants received only 100 ml of water per percolator per day for a period of 10 days, thereby allowing time for nematode entry into roots.

Nutrient solutions in distilled water were prepared from stock solutions of the following C. P. salts: $Ca(NO_3)\cdot 4H_2O$; KH_2PO_4; K_2SO_4; $NaH_2PO_4\cdot H_2O$; $CaCl_2\cdot 2H_2O$; KCl; $NaNO_3$; $CuSO_4\cdot 5H_2O$; $FeSO_4\cdot 7H_2O$; $MgSO_4\cdot 7H_2O$; $MnSO_4\cdot 4H_2O$; $H_2MoO_4\cdot H_2O$; $ZnSO_4\cdot 7H_2O$ and H_3BO_3. The composition of the nutrient solutions supplied are shown in Table 1.

To avoid serious depletion, nutrient solutions were renewed as often as necessary. The frequency of renewal was 5 to 10 days, depending on the solution volume in relation to plant growth and environmental conditions. The pH of nutrient solutions varied between 5 and 5.4.

The experimental design was a complete randomized block of 10 treatments with three

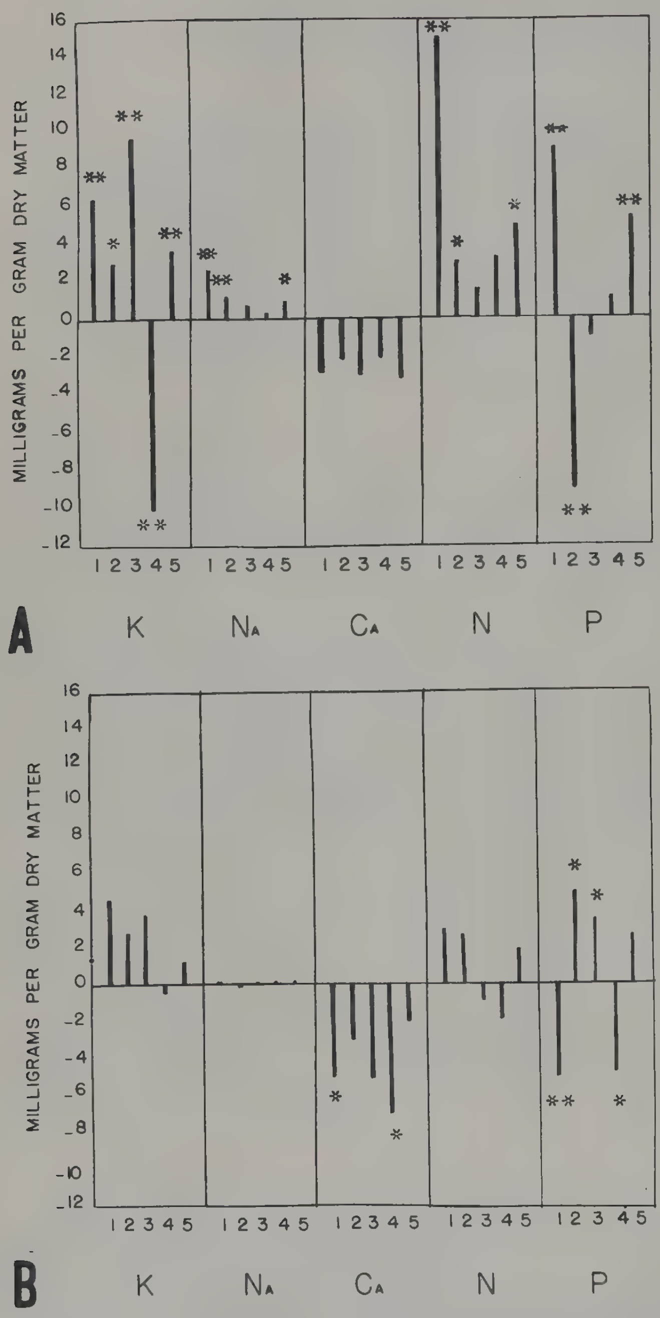

FIGURE 1. Relative intensity of internal nutrient accumulation of elements in roots (A) and tops (B) of pepper plants infected with Pratylenchus penetrans under five different treatments. 1) complete nutrient, 2) K-deficient, 3) N-deficient, 4) P-deficient, 5) Ca-deficient. *Indicates significant difference at 5% level. ** Indicates significant difference at 1% level.

replications of three plants each. During the course of the experiment greenhouse temperatures varied from 21° to 32° C.

Six weeks after inoculation, plants were removed and their roots washed free of sand. Fresh weights of tops and roots were determined. Plant material was dried and analyzed for phosphorus, nitrogen, sodium, potassium, and calcium. Phosphorus was determined by the official A. O. A. C. method (4). Total nitrogen concentrations were determined spectrographically after the method of Lindner (5). Potassium, sodium, and calcium concentrations were determined with a Beckman flame spectrophotometer, following standard spectrophotometric methods.

RESULTS AND DISCUSSION

Inoculated and uninoculated plants were treated with the five different nutrient solutions listed in Table 1. Except for plants provided with a calcium-deficient solution, all plants with nutrient solutions deficient in a single element were retarded in growth (Table 2). That calcium deficiency symptoms failed to develop was possibly due to the presence of quantities of calcium in the sand culture adequate for normal growth of pepper plants. The average fresh weight of infected plants varied from the average fresh weight of its corresponding control (Table 2). Such variation was due to retarded growth in all treatments with the exception of phosphorus-deficient plants, in which growth of inoculated plants was stimulated by nematodes. Although differences in average weight between inoculated and uninoculated plants were not great in some treatments, analyses of variance revealed that such differences were significant at the 5% level of probability.

Results (Table 2) indicate that host nutrition influenced the severity of pathogenic effects and the degree of retardation in plant growth following nematode infection. Phosphorus-deficient plants were stimulated with a relatively small number of nematodes, whereas potassium-, calcium-, and nitrogen-deficient plants were markedly stunted as a result of nematode infection. Stunting was most pronounced in plants deficient in nitrogen.

Results of analyses of tops and roots of inoculated and uninoculated plants under five different nutrition conditions are summarized in Figures 1A and B. Most of the changes in element concentrations which resulted from nematode infection occurred in the roots.

In roots, potassium concentrations were the most affected by nematode infection, whereas calcium concentration did not change under any of the five different nutritional conditions (Fig. 1A). Except for plants provided with a phosphorus-deficient solution, potassium accumulated in the roots of all other treatments as a result of nematode attack. Sodium, nitrogen, and phosphorus concentrations of these elements varied from those of checks only in complete, K-deficient, and Ca-deficient treatments. Potassium decrease in infected phosphorus-deficient plants and phosphorus decrease in infected potassium-deficient plants is evidence of abnormal internal nutrient accumulation of elements resulting from nematode injury.

Little change in mineral concentrations between infected and check plant tops was observed (Fig. 1B). Calcium decrease was observed in infected plants treated with complete and phosphorus-deficient solutions. Phosphorus was also reduced on these same treatments.

Stimulation of the growth of phosphorus-deficient plants following nematode infection was probably due to nutritional conditions resulting from nematode parasitism in this particular treatment. Chitwood, et al. (2) observed similar results when they found that moderate quantities of Meloidogyne incognita and M. javanica sometimes caused significant increases in peach growth.

Because of the diversity in results, further work in the area of plant nutrition, as influenced by parasitic nematodes, needs to be conducted. Possibly the most suitable data will result from tests conducted under controlled environmental conditions.

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EFFECTS OF CERTAIN MIXTURES OF FUNGICIDES AND SPRAY ADDITIVES ON THE CONTROL OF PEACH SCAB AND BROWN ROT[1]

W. A. Chandler[2]

Abstract

Four years of spraying Elberta peach trees with captan and mixtures of captan with various additives showed that when the fungicides glyodin and dodine were added to captan, the control of brown rot and scab was equal to or better than the control provided by captan alone. The addition of Plyac or Ortho Spray Sticker resulted in less effective control of brown rot than that obtained with captan alone, in two successive years. In a single test against peach scab both additives resulted in improved control.

INTRODUCTION

In the Southeast the two principal peach diseases that are subject to control by the regular spray schedule are brown rot, caused by *Monilinia fructicola* (Wint.) Honey, and scab, caused by *Cladosporium carpophilum* Thuem. Sulfur and captan are the fungicides generally recommended. Under ordinary conditions, either fungicide usually provides satisfactory control of these two diseases, but under severe conditions sulfur is not so effective against brown rot as might be desired. Captan is quite effective against brown rot but not highly resistant to weathering. It is often difficult and always expensive to make the extra spray applications needed in rainy weather.

The possibility of improving disease control by adding a "sticker" to captan or certain other fungicides has been recognized for some time, and a number of commercial additives are available. Frequently chemical residue analyses are cited as evidence that a particular additive is effective. Unless disease control is improved or at least not impaired, a "sticker" is biologically ineffective, regardless of residues.

Another possibility for improving disease control involves mixing two or more fungicides. Daines (2) reported the advantages and disadvantages of a number of fungicide mixtures, as observed by him and other workers on several crops including peaches. None of these mixtures included a fungicide with surfactant properties, but in a later paper (3) he did report on one such mixture.

In the experiments described here several mixtures of captan with various additives, including surfactant fungicides, were tested.

MATERIALS AND METHODS

The following six fungicides and four spray additives were used in one or more of a series of field spraying experiments between 1958 and 1961 at the Georgia Experiment Station.

Chemical Name	Common Name	Trade Name
N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide	captan	(Orthocide-50)
N-dodecylguanidine acetate	dodine	(Cyprex-65W)
N-dodecylguanidine acid phthalate		(EF 23441 70%)
2-heptadecyl glyoxalidine acetate	glyodin	(Crag 30% soln.)
Sulfur	sulfur	(Nutonex 94% sulfur)
Tetramethylthiuram disulfide	thiram	(Thylate 65%)
Alkyl aryl polyoxyethylene glycols, free fatty acids, isopropanol		(Multifilm X 77 spreader activator)
Emulsifiable AC polyethylene fatty acid condensate, alkyl sulfonate		(Plyac Spreader Sticker)
Not available		(Ortho Spray Sticker)
Sodium sulfates of mixed long chain alcohol - fatty acid esters, diethylene glycol abietate		(DuPont Spreader Sticker)

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[1] Paper No. 417, Journal Series, Georgia Experiment Station, Experiment, Georgia.

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Thiram has been included as an approved peach fungicide in recent spray schedules and, like captan, it might conceivably provide better disease control with the addition of a suitable material to improve its resistance to weathering. Glyodin has been cleared by the Food and Drug Administration for use on peaches. Dodine has been cleared for use on several deciduous fruits, not including peaches. Both glyodin and dodine were used in the spraying tests here reported, mainly as additives. Like several of the commercial spray additives included in these experiments, glyodin and dodine contain long chain fatty acids which give these materials surfactant properties. Chandler (1) reported the first use of a captan-glyodin mixture on peaches. Diener and Carlton (4) first reported on the effects of a dodine-captan mixture in controlling the bacterial spot disease. They observed apparent synergism and safening of dodine by captan. Kretchman and Mitchell (5) show in a table of surface-tension data that glyodin reduced the surface tension of water as much as DuPont Spreader Sticker when each was used at the same concentration. The fungicide designated as EF 23441 differs from dodine in that it is the acid phthalate instead of the acetate. It was tested as a new fungicide in 1958 and 1959 and again in 1960 and 1961. Only in 1961 was this material mixed with captan.

Except in 1958, the amount of captan or thiram was reduced from 2 pounds to 1 1/2 pounds when 1 pint of glyodin or 1/2 pound of dodine was added. The fungicides were used at the full rate with non-fungicidal additives.

Except for one experiment in a grower's orchard in 1958, all of the fungicidal tests were conducted in a planting of Elberta, Southland, and Coronet peach trees at the Georgia Experiment Station. A high pressure sprayer with a 12,500 ft^3/min blower was used. Elberta trees were sprayed 7 to 10 times starting with petal fall, the earlier varieties not receiving the last one or two applications. Plots consisted of three or four trees, and four replications were used in each test. Data on peach scab were taken at harvest. The brown-rot data were taken from samples of 100 peaches per plot, free of rot when picked and stored for several days.

In 1958 peaches were held at room temperature. In subsequent years refrigerated chambers were used. In 1961, at the lower storage temperature of 65° F, Rhizopus rot development was delayed more than brown rot, making it easier to evaluate control of brown rot.

Tables 1-4 give disease control results on the Elberta variety.

RESULTS

Tables 1 and 2 are arranged in the order of scab control since there were highly significant differences in the control of this disease both in 1958 and in 1959 on the Elberta variety. In both years the best treatment was EF 23441 and with captan plus glyodin ranking second best. Adding glyodin to thiram in 1958 or to sulfur in 1959 did not result in improved disease control. In a 1959 test on the Coronet variety, scab control was similar to that on Elberta with captan

Table 1. Control of peach scab and brown rot - 1958.

Treatment (lb/100 gal.)[a]	% of fruit diseased		
	scab[b, c]	brown rot at harvest	brown rot after 3 days at 90° F[b, c]
EF 23441 1 1/2	0.5 A	3.4 A	23.3 A
Captan 2 + glyodin 1 pt	3.8 A	1.7 A	16.1 A
EF 23441 3/4	4.0 A	2.2 A	10.8 A
Captan 2	21.5 B	2.4 A	31.1 A
Thiram 2 + glyodin 1 pt	26.5 B	11.2 A	46.5 A
Thiram 2	32.8 B	5.7 A	33.6 A
Check (no fungicide)	89.3 C	7.4 A	37.0 A

[a] Sprays applied

	Apr. 9,	17,	29;	May 9,	22;	June 5,	23;	July 11.
Inches of rain between sprays	2.8,	0.6,	0.6,	1.8,	0.0,	1.5,	4,8,	2.0

[b] Percentages converted to angles for calculations.
$\text{Angle} = \arcsin \sqrt{\text{percentage}}$

[c] Percentages followed by the same letter do not differ significantly at the .05 level according to Duncan's Multiple Range Test.

Table 2. Control of peach scab and brown rot - 1959.

Treatment (lb/100 gal.)[a]	% of fruit diseased: scab[b, c]	% of fruit diseased: brown rot after 5 days at 70° F[b, c]
EF 23441 1	2.5 A	23.0 A
Captan 1 1/2 + glyodin 1 pt	8.5 A B C	21.5 A
Captan 2	12.5 B C D	21.3 A
Sulfur 6	15.3 B C D E	14.3 A
Sulfur 4 1/2 + glyodin 1 pt	15.3 B C D E	17.0 A
Glyodin 1 qt	35.3 E F	24.0 A
Glyodin 1 pt	54.5 F	20.5 A
Check (no fungicide)	99.8 G	32.0 A

[a]Sprays applied

	Apr. 1,	11,	20;	May 2,	18;	June 3,	10,	24;	July 9.
Inches of rainfall between sprays	1.5,	1.7,	0.5,	1.4,	9.5,	2.5,	0.6,	1.8,	1.7

[b]Percentages converted to angles for calculations. Angle = arc sin $\sqrt{\text{percentage}}$

[c]Percentages followed by the same letter do not differ significantly at the .05 level according to Duncan's Multiple Range Test.

Table 3. Control of brown rot - 1960.

Treatment (1b/100 gal.)[a]	% of fruit diseased after 7 days, 75°-85° F[b, c]
Captan 1 1/2 + dodine 1/2	0.2 A
Captan 2	0.7 A B
Captan 1 + dodine 1	1.0 A B C
Captan 1 1/2 + glyodin 1 pt	2.4 A B C D
EF 23441 1	3.3 A B C D
Sulfur 6	7.7 B C D E
Sulfur 6 + glyodin 1 pt	9.6 C D E
Captan 2 + Ortho Spray Sticker 8 oz	9.8 C D E
Captan 1 1/2 + dodine 1/2 + Ortho Spray Sticker 8 oz	10.8 D E
Dodine 1	11.1 D E
Check (no fungicide)	12.0 D E
Captan 2 + Plyac 4 oz	13.0 E
Captan 2 + Multifilm X 77, 3 oz	16.4 E

[a]Spray applied

	Apr. 9,	21;	May 4,	17;	June 3,	22;	July 8.
Inches of rainfall between sprays	0.6,	0.4,	0.9,	0.6,	0.4,	0.6,	0.7

[b]Percentages converted to angles for calculations. Angle = arc sin $\sqrt{\text{percentage}}$

[c]Percentages followed by the same letter do not differ significantly at the .05 level according to Duncan's Multiple Range Test.

Table 4. Control of scab and brown rot - 1961

Treatment (lb/100 gal.)[a]	% of fruit diseased: scab at harvest[b, c]	% of fruit diseased: brown rot after 6 days at 65° F[b, c]
Captan 1 1/2 + dodine 7 oz	0.1 A B	1.6 A
Captan 2	5.1 D E F	4.3 A B C
Captan 1 1/2 + glyodin 1 pt	0.5 A B C	5.9 A B C
Captan 1 1/2 + EF 23441 1/2	1.4 A B C D E F	6.7 A B C
Thiram 1 1/2 + glyodin 1 pt	5.9 E F	10.0 A B C
Captan 2 + Ortho Spray Sticker 1/2 pt	0.0 A	10.1 A B C D
Sulfur 6	2.2 B C D E F	10.3 A B C D
Thiram 2 + DuPont Spreader Sticker 1 1/2 oz	5.0 D E F	11.9 B C D
Thiram 2	3.5 C D E F	12.1 B C D
Captan 2 + Plyac 4 oz	1.0 A B C D	15.0 C D
Check (no fungicide)	61.1	25.0 D

[a]Sprays applied

Sprays applied	Mar. 22;	Apr. 4,	18;	May 8,	18,	29;	June 16,	23;	July 3,	14.
Inches of rainfall between sprays	4.1,	3.5,	2.8,	2.9,	3.9,	.8,	3.7,	1.2,	1,8,	.54

[b]Percentages converted to angles for calculations. Angle = arc sin $\sqrt{\text{percentage}}$

[c]Percentages followed by the same letter do not differ significantly at the .05 level according to Duncan's Multiple Range Test.

plus glyodin providing slightly better control than EF 23441 or captan alone, all at the same dosage rates as used on Elberta. The captan-glyodin treatment also gave the best control of brown rot on the Coronet variety and was significantly better than sulfur or EF 23441 but not significantly better than captan. Differences in brown-rot control on Elberta as shown in Tables 1 and 2 were not significant in 1958 or 1959.

Chemical residue analyses for captan on Elberta peaches in 1959 showed substantially less captan remaining 20 days after the last application where glyodin had been added than where captan alone had been applied.

Tables 3 and 4 are arranged in order of brown-rot control since there were significant differences in control of this disease in 1960 and 1961 in the same block of Elberta trees to which the first two tables pertain. The best treatments for brown-rot and scab control were similar except that the captan-dodine mixture had not been used in 1958 or 1959. Dodine alone at 1 pound was ineffective against brown rot in 1960. The closely related EF 23441, however, was effective against brown rot in 1960. Table 2 shows that glyodin alone was ineffective against peach scab in 1959. By 1961 the EF 23441 used was 2 years old and perhaps had broken down somewhat, but disease control with the mixture was still fairly good.

Although no appreciable phytotoxicity resulted from the use of dodine alone or in mixtures with captan on Elberta trees in 1960 or 1961, there was severe defoliation on the Southland variety in 1961 where 1/4 pound of dodine was used with 3 pounds of captan. This injury followed one application which dried very slowly due to light rain.

The most striking results in Tables 3 and 4 are the sharply reduced control of brown rot when the commercial additives were used with captan. All were used at rates recommended by the manufacturers. The addition of Ortho Spray Sticker, Plyac, or Multifilm X 77 to captan resulted in significantly more brown rot than with captan alone in 1960. In 1961 there were fewer significant differences, but the results were essentially the same so far as brown-rot control was concerned. It had been expected that the stickers might perform better in 1961, which was a wet season, than in 1960 which was very dry during the period when sprays were being applied. It does appear that Ortho Spray Sticker helped provide perfect control of scab in 1961. Captan with any of the additives used provided better scab control than captan alone. There was no scab except on unsprayed trees in 1960.

In a spraying test in a grower's orchard in 1958 Ortho Spray Sticker added to captan also resulted in better scab control but poorer brown-rot control.

DISCUSSION

Some of these results are difficult to explain, particularly with the commercial additives which resulted in reduced brown-rot control in two experiments but in the 1961 test resulted in improved scab control.

Miller and Stoddard (6) reported a similar loss of control of grey mold of strawberry when Ortho Spray Sticker was added to captan. Other workers have reported improved control of certain diseases by adding either Plyac or Ortho Spray Sticker to various fungicides.

The manufacturers of both glyodin and dodine claim on their label or in technical information that these surfactant fungicides become redistributed by washing and dripping of rain. In the case of glyodin this claim is based in part on the work of Thurston, et al. (7), who found that apple scab was controlled on new leaves which had developed after the final fungicide application. It seems likely that both glyodin and dodine may help to maintain an effective coverage of peach fruits more by tending to redistribute the spray residue during rains than by resisting removal of residues as some of the commercial "stickers" apparently function. Various workers have shown that in the Southeast peach-scab infection occurs mainly at 4 to 6 weeks after petal-fall, when fruits are not expanding very rapidly. Brown-rot infection takes place at a later period, close to harvest, when fruit expansion is very rapid. During the preharvest period, redistribution may be more important than maximum retention of fungicidal residues.

CONCLUSIONS

Under the conditions of the spraying experiments reported here, evidently several commercial spray additives reduced the effectiveness of captan against brown rot. The surfactant fungicides dodine and glyodin did not reduce the effectiveness of captan, and in some instances control of either brown rot or scab was more effective with a mixture of dodine-captan or glyodin-captan than with captan alone. Owing to the severe phytotoxicity resulting in one test from the dodine-captan mixture, apparently this mixture cannot be recommended for general use. The glyodin-captan mixture could be recommended for use on a trial basis, being both safe and effective.

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GEORGIA EXPERIMENT STATION, EXPERIMENT, GEORGIA

A NEW WILT DISEASE OF MAIZE IN EGYPT[1]

A. S. Samra, K. A. Sabet, and M. K. Hingorani[2]

In 1960 an apparently undescribed disease of maize was observed in Egypt (1). A survey made that year of the principal maize-growing areas showed it to be widespread, with an incidence ranging to 25% in some fields (2).

SYMPTOMS

The first symptom of this disease is a moderately rapid wilting of plants usually at about time of flowering, although the onset of this symptom may occur from just prior to flowering until shortly before maturity. Wilting progresses from lower to upper portions of the plant. Leaves first become dull green, eventually lose color, and become dry as though suffering from lack of water. Vascular bundles in the stalk become reddish brown; and within a short period of time, lower internodes assume this color. In advanced stages of the disease, lower portions of the stalk become dry, shrunken, and hollow. After the first wilt symptom appears, progress of the disease is relatively rapid. Because of the delay in appearance of initial symptoms until about flowering, this disease has been designated as "late wilt."

Stalk symptoms may be modified by secondary invasion by saprophytic organisms. Often in such cases the lower internodes become watery. This condition is called "wet rot." The infection may sometimes be accompanied by Erwinia lathyri, and in such instances lower internodes become soft and wet.

ISOLATION OF THE CAUSAL ORGANISM AND CONFIRMATION OF PATHOGENICITY

A number of fungi and bacteria have been isolated from maize stalks at different stages of infection. One fungus isolate, based on preliminary inoculation experiments made in Egypt, appeared to be capable of inciting symptoms typical of those found in the field.

Further studies conducted in the greenhouse at Purdue University confirmed the pathogenicity of this fungus. The double-cross hybrid Ind. 620 (WF9 x Hy) x (Os 420 x M 14) was grown in Perlite (an expanded silica) and fed a nutrient solution (Hoagland and Arnon's #1). Inoculations were made by seeding the substrate with a suspension of spores and mycelial fragments of the fungus at the time of planting or by introducing the suspension into the root zone 1 month after emergence of the seedlings. Temperature in the greenhouse ranged from about 15° to 28°C.

Care was taken to confine the pathogen by collecting drainage from pots in a concentrated solution of $CuSO_4$ and by autoclaving plants, pots, and substrates at the end of the experiment.

Symptoms began to appear in some plants at about full silk, and subsequently in others until the experiment was discontinued 4 weeks later. The symptoms were generally similar to those described above as they appear in the field. Plants inoculated with Diplodia maydis, Gibberella zeae, or Pythium aphanidermatum showed no wilting during the course of the experiment. Non-inoculated controls remained free from symptoms (Fig. 1).

Histological preparation of roots and stalks of wilted plants showed spores and mycelium typical of the pathogen to be present in the xylem vessels (Figs. 2 and 3). The fungus was readily recovered when stalk and root tissues were planted on potato-dextrose agar.

A rapid method to test the virulence of the pathogen was conducted, wherein maize seed was planted in "Perlite," in sand, or on water agar in large tubes or beakers. These substrates were seeded with the pathogen prior to planting. The pathogen was capable of causing severe reduction in root growth, and the roots showed brown discoloration. The aerial portions of the plants so affected showed no symptoms after 1 1/2 months when the experiments were discontinued. One isolate of the pathogen appeared to be non-virulent and had no effect on root

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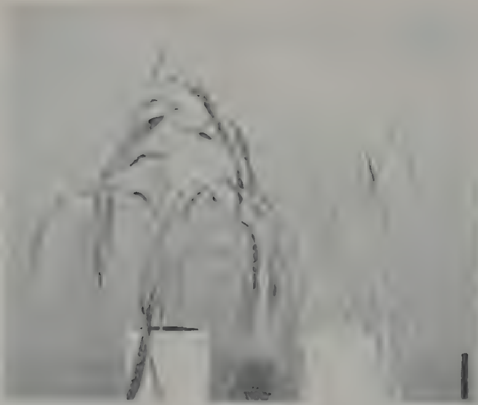
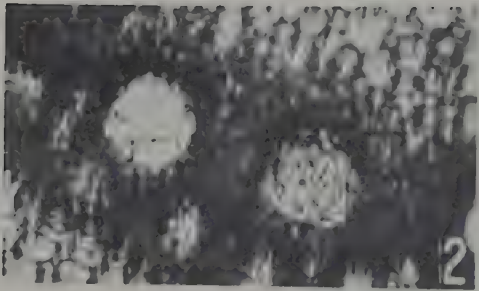
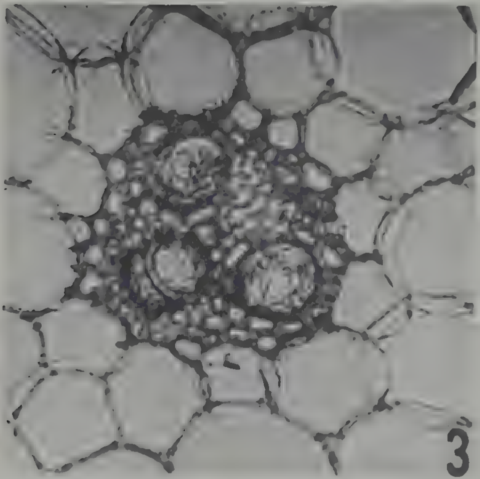
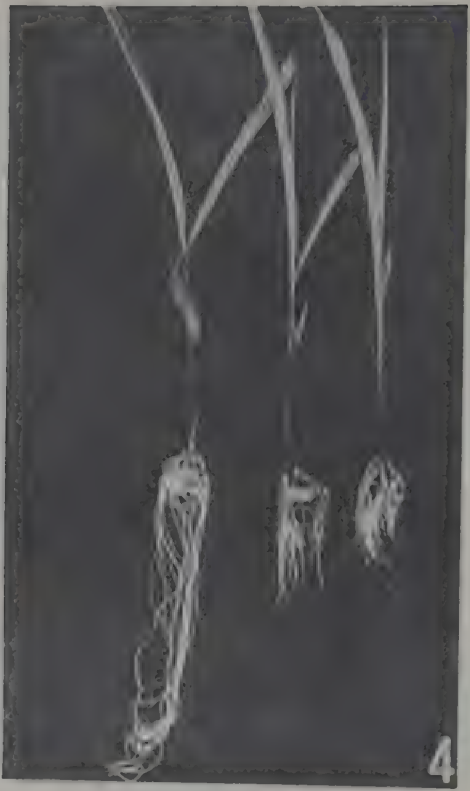
[1] The authors are grateful to Dr. A. J. Ullstrup for counsel and advice during that part of the research conducted by the senior author while an F. A. O. Fellow (Andre Mayer Research Fellowship) in the United States, and to Dr. J. R. Shay, Head of the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana for providing space and facilities during this period.

[2] Director, Maize Diseases Project, Plant Pathology Department, Ministry of Agriculture, Giza-Orman, Cairo, Egypt; Assistant Professor of Plant Pathology, University of Cairo, Giza, Cairo, Egypt; and F. A. O. Maize Pathologist, Plant Pathology Department, Ministry of Agriculture, Giza-Orman, Cairo, Egypt, respectively.









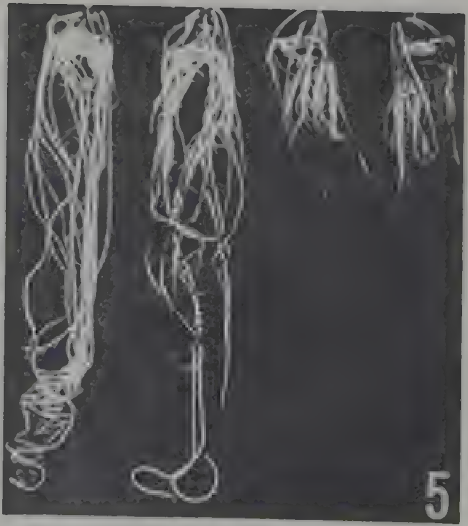


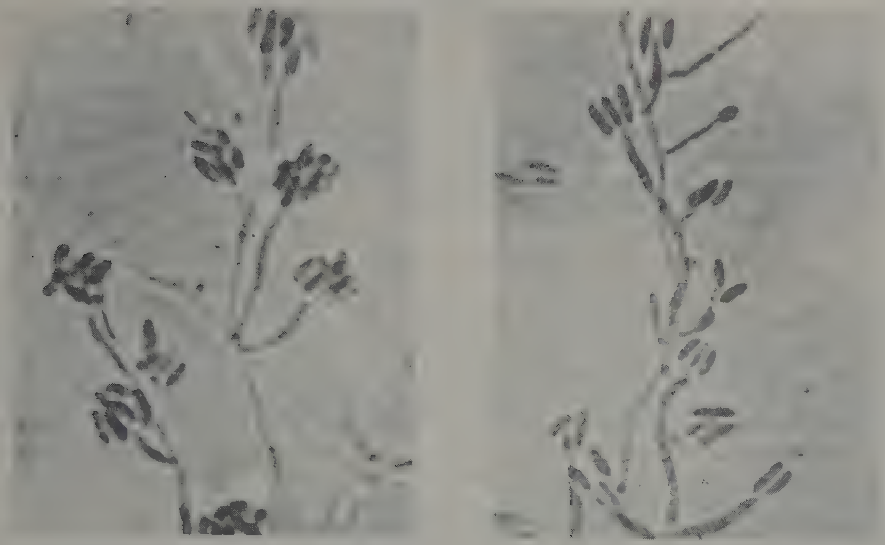
FIGURE 1. At right, typical wilt symptom resulting from artificial inoculation of plants grown in nutrient solution in the greenhouse. At left, noninoculated control.

FIGURE 2. Cross section of infected maize stalk showing mycelium in xylem vessels.

FIGURE 3. Cross section of infected maize root showing mycelium in xylem vessels.

FIGURE 4. Reduction in root development of inoculated maize seedlings compared with noninoculated control.

FIGURE 5. Variation in virulence of cultures. From left to right, noninoculated control, nonvirulent isolate of the pathogen, and virulent isolates showing effect on root growth.



FIGURES 6 and 7. Photomicrographs of the pathogen showing the formation of clusters of spores.

growth (Figs. 4 and 5).

Preliminary experiments with the pathogen have shown that it grows well and sporulates on a number of the common vegetable-extract agars such as potato-dextrose, oatmeal, yeast extract-glucose, and prune. Conidiophores are mostly branched with conidia forming successively and exogenously at the apices so that several spores may collect in heads (Figs. 6 and 7). On potato-dextrose agar the conidia are hyaline, single-celled, oblong, and measure 3.6-14 x 3-3.6μ, averaging 7.2 x 3.5μ. Colonies are low-growing and felty, at first white but turning gray with age. Some cultures become slate gray to black after 2 to 4 weeks of growth. The margin of the colony is "rhizoid" and hyphal ropes are often oriented in a clockwise direction.

On the basis of morphology, the fungus appears to be a species of *Cephalosporium*. The colony characters are distinct from *C. acremonium*, *C. gregatum*, and *C. gramineum* with which it has been compared. The optimum temperature for growth of the pathogen on potato-dextrose agar is about 30°C, with the minimum at 10° and the maximum at 36°. Further studies concerning identification of the parasite and host range are being conducted.

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SOME OBSERVATIONS ON THE NUMBER OF ROOT LESION NEMATODES NECESSARY TO CAUSE INJURY TO SEEDLING ONIONS[1]

John M. Ferris[2]

Summary

Observations of onions growing in muck soils infested with *Pratylenchus penetrans* (Cobb) were carried out in northern Indiana. Evidence is presented to indicate that when more than 100 *P. penetrans* are present per gram of onion root, seedlings show characteristic symptoms of nematode injury.

INTRODUCTION

In Indiana, bulb or dry onions grown from seed in muck soils sustain severe nematode injury, especially in early spring, from attack by the root lesion nematode, *Pratylenchus penetrans* (Cobb). Growers are rapidly learning that in order to grow onions when this nematode is present, the soil must be fumigated to reduce the nematode populations. Although this disease was discovered in 1947[3] little information is available as to how many nematodes are necessary to injure onions. Observations were made during the past growing season in three muck fields in northern Indiana. One field was located near Newland, Indiana and the other two were 55 miles northeast of Newland, near North Liberty, Indiana.

A large field of muck soil near Newland, approximately 200 acres in size, was treated by the grower on October 14, 1960 with dichloropropenes after a successful potato crop had been harvested. Half the field was treated with 1,3-dichloropropene and 1,2-dichloropropane (D-D) at the rate of 45 gallons/acre and the other half with 1,3-dichloropropene (Telone) at the rate of 40 gallons/acre. Samples were collected by the author from the field in December 1960 and in May and June 1961. Onion seed, principally Downing Yellow Globe, was planted in this field during March and April 1961. By late spring, onions showing characteristic symptoms of nematode injury were evident in some scattered areas over the field. These areas, all in the early planted onions, ranged mainly from 1/4 to 1 acre in size, with one extensive area 25 acres in size.

Muck soil and onion root samples were also collected from two onion fields near North Liberty in May 1961. In one field, there was a general yellowing and stunting of onion plants with only a small portion of the field having good plants. In the other field, the growth of the onions was judged to be normal and healthy.

MATERIALS AND METHODS

Soil samples were processed in the following manner. A 500-cc aliquot of soil was taken from each muck soil sample, 1 to 2 quarts in size, after it had been carefully mixed. Nematodes were removed by a combination decanting and sieving process. Residue caught on the #200 mesh screen was placed on tissue paper in a wire basket of a Baermann funnel and the nematodes drawn off after 24 and 48 hours. Onion roots were washed, blotted on paper toweling, weighed and placed in a quart jar with a small amount of water. Nematodes were collected at 24 and 96 hours, at which time the roots were decomposing.

RESULTS

The population of *P. penetrans* in the Newland field was estimated from fall samples obtained in surrounding fields to have been between 300 and 600 nematodes per 500 cc of soil prior to fumigation. The December samples indicated the population had been reduced to 32 to 65 root lesion nematodes per 500 cc. Nematode populations in soil and onion roots from areas of stunted and healthy appearing plants were compared (Table 1).

The numbers of nematodes recovered (Table 2) from the soil and roots of healthy and diseased plants from the two North Liberty fields were remarkably similar to the Newland samples.

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[1] Journal Paper No. 1923 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.
[2] Assistant Professor, Department of Entomology, Purdue University, Lafayette, Indiana.
[3] Unpublished Dow Chemical Co. Field Agricultural Chemicals Research Report.

Table 1. *Pratylenchus penetrans* counts from onion root and muck soil samples collected around normal and stunted onion seedlings in a field near Newland, Indiana, 1961.

Date of collection	Condition of plants	Nematodes per gram of onion root	Nematodes per 500 cc of soil
May 16	normal[b]	278	5
May 16	stunted[b]	1800	33
May 19	normal[a]	46	1
May 19	normal[b]	100	1
May 19	stunted[b]	683	14
June 8	normal[b]	3	0
June 8	(stunted)[c]	80	19

[a] Seed planted April 26.

[b] Seed planted March 28.

[c] Onions in this portion of the field were disked under several days prior to sampling.

Table 2. *Pratylenchus penetrans* counts from onion root and muck soil samples collected around normal and stunted onion seedlings in two fields near North Liberty, Indiana, 1961.

Field	Date of collection	Condition of plants	Nematodes per gram of onion root	Nematodes per 500 cc of soil
A	May 18	yellowed, stunted	354	--
A	May 18	yellowed, stunted	485	--
B	May 29	normal	35	5
A	May 29	normal	25	24
A	May 29	yellowed, stunted	242	63
A	May 29	yellowed, stunted	153	47
A	May 29	slightly stunted	1200	127

With few exceptions, onion plants which showed normal growth contained fewer than 100 *P. penetrans* per gram of root (Tables 1 and 2). The number of root lesion nematodes in injured plants ranged from 153 to 1800 per gram of root. It is interesting to note that, with one exception, more than five individuals of *P. penetrans* per 500 cc of soil resulted in injury to the onions.

DISCUSSION

The areas of poor onion growth in the 200-acre Newland field were so scattered and irregular in shape that it did not appear that either the fumigation application or the action of the chemicals could be blamed for these failures. Since the 1961 spring was cold for a very long period and snow covered the field following the March 28 planting before the onion seed had germinated, weather seemed to offer a possible explanation for the nematode damage. The fact that there were no nematode injured onions in the late planted (April 26) portion of the field supports this hypothesis. Work is currently in progress to determine whether there is a soil temperature at which the growth of onion roots is essentially stopped but feeding and reproduction of *P. penetrans* continues.

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TARGET BLOTCH OF SUGARCANE IN FLORIDA

Edwin H. Todd[1]

Target blotch, caused by Helminthosporium sp., was found on leaves of sugarcane plants, Saccharum officinarum, at Canal Point, Florida in January 1959. This appears to be the first record of target blotch in the United States. In 1929 the disease was reported in Cuba by Priode (1), who later described it in detail (2). Since Priode's publications, target blotch has been reported from the Union of South Africa (3).

Infections occur only on leaves. They are characterized by straw- to brownish-colored necrotic areas having irregular, concentric rings, which roughly resemble a target board in appearance (Fig. 1). The rings develop first on the young rolled leaves of the leaf spindle, but the target board effect is more pronounced after the leaves unroll. The necrotic areas may be few or many and may involve sizable portions of the leaf, because of the coalescing of adjacent infections.

Although the causal fungus is typically a Helminthosporium, the identity of the species has not yet been determined. Also, no other host for the target blotch fungus has been reported. The disease occurs infrequently at Canal Point during the winter and is considered economically unimportant.

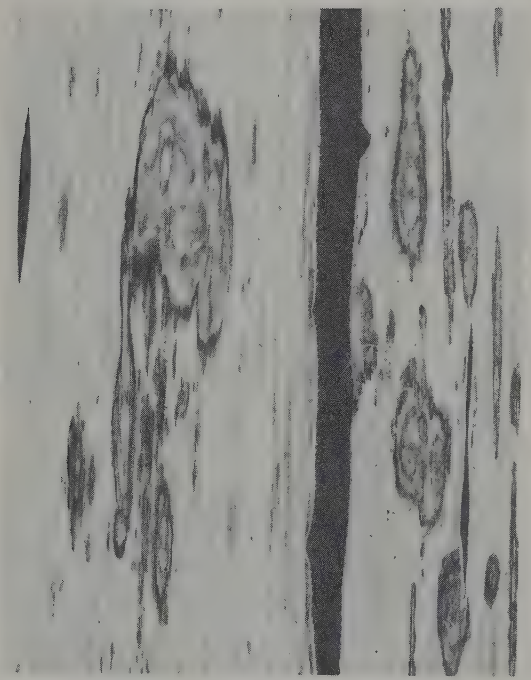

FIGURE 1. Target blotch of sugarcane. Infected leaves of clones Hawaiian Original 52, left, and D. 74, right.

The writer wishes to thank C. R. Benjamin for confirming identity of the disease.

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TEMPERATURE FACTORS IN THE CONTROL OF BLACK SPOT OF CITRUS[1]

Roy J. Smith[2]

Abstract

Black spot is a serious problem in the handling of South African citrus. Low temperatures are recognized as a significant control factor. The argument presented here is that such controls are practical possibilities in South Africa without resort to expensive mechanical refrigeration.

THE PROBLEM

Black spot commonly occurs as a postharvest disease and is characterized by major expression at temperatures above 70°F, particularly above 80° (1). One of the most important examples of black spot development as a postharvest disease is found in the shipment of citrus from the northern Transvaal area of South Africa to Capetown, a railway journey of 1000 to 1200 miles that usually requires about a week (Fig. 1). Temperatures in September-October usually rise above 70° (Tables 1 and 2). (The average of September and October is chosen as best representing the temperatures at the end of the shipping season. The combination at this time of high temperatures and maximum maturity for the fruit makes it very susceptible.) However, high temperatures may occur any time during the year.

The railway cars have louvered sides and ends and the containers are slatted; consequently, air penetrates directly to the fruit. The fungi are in the outer portion of the rind, in a position highly susceptible to temperature changes. Development of the fungi during shipment often is rapid and widespread.

THE PROPOSED SOLUTION

Black spot, as a postharvest disease, apparently can be controlled by moderate temperatures. Calavan reports that "Cool temperature storage is the only known practical means for effectively controlling the postharvest phase of black spot. Appearance of postharvest lesions

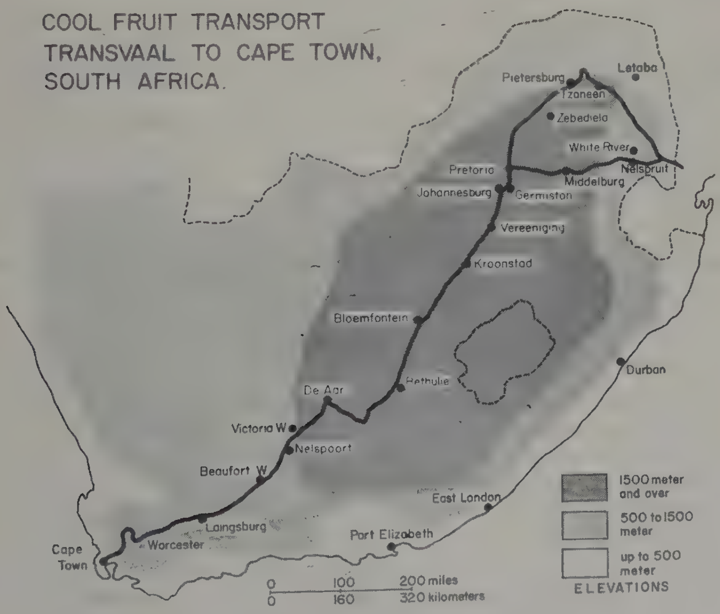
COOL FRUIT TRANSPORT
TRANSVAAL TO CAPE TOWN, SOUTH AFRICA.
Pietersburg
Letaba
Tzaneen
Zebediela
White River
Nelspruit
Pretoria
Middelburg
Johannesburg
Germiston
Vereeniging
Kroonstad
Bloemfontein
Bethulie
Durban
De Aar
Victoria W.
Nelspoort
Beaufort W.
East London
Laingsburg
Worcester
Port Elizabeth
Cape Town
0 100 200 miles
0 160 320 kilometers
1500 meter and over
500 to 1500 meter
up to 500 meter
ELEVATIONS

FIGURE 1.

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[1] This analysis was developed while the author was serving in a consulting capacity with the South African Cooperative Citrus Exchange.

[2] Professor of Agricultural Economics, Department of Horticulture, University of California, Riverside.

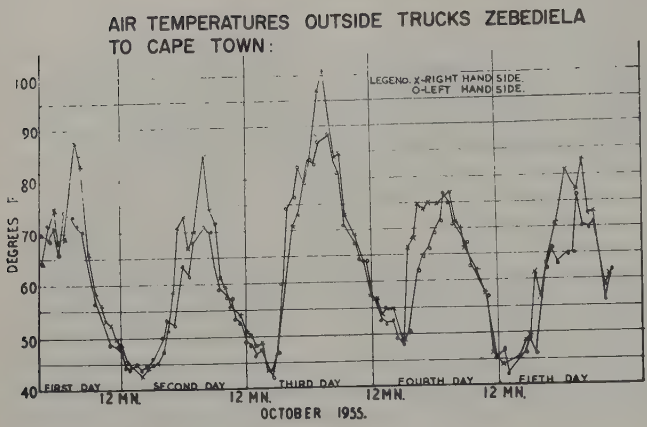

FIGURE 2.

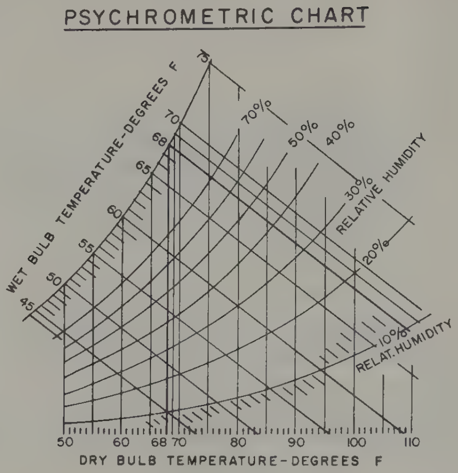

FIGURE 3.

is almost completely suppressed by storing fruit below 70°F" (1).

A common comment about the problem is that the solution is to be found in mechanical refrigeration, but that it is too expensive. The point to be made here is that desirable temperature conditions probably can be provided without resort to mechanical refrigeration and without great expense.

Night temperatures en route from the Transvaal to Capetown are very desirable, well below 70°F (Tables 3 and 4). The contrast between afternoon and night temperatures is shown in Figure 2. The problem then becomes one of countering the high afternoon temperatures. The methods suggested are as follows:

1. Insulation: The fruit is to be carefully protected from direct influence of the sun or of hot winds. Expensive refrigeration insulation is not needed, however, since it is not proposed to keep the fruit at very low temperatures.

2. Night Cooling: If the night air is cooler than the fruit, this air is moved through the fruit.

To keep out hot winds but to allow in cool air will require some method of controlling air movement through the railway cars.

3. Evaporative Cooling: Where humidities are low, evaporative techniques have long been established as a practical inexpensive method of cooling fruit. The possible changes in temperature are indicated by the psychrometric chart shown in Figure 3. "In practice, a temperature within about 5% of the wet bulb depression above the wet bulb can be readily reached"[3].

It seems probable that evaporative cooling will be necessary during the warm part of the day. A study of the relative humidities prevailing during the afternoons (Tables 5 and 6) and of the psychrometric data in Figure 3 indicates it is quite practical to keep air temperatures at a safe level, almost always well below 70°F. The prime objective of the evaporative cooling will be to keep a layer of cool air between the roof and walls of the railway car and the fruit.

Cooling the fruit initially is the major problem. In September-October the production areas where the packing houses are located have high dangerous temperatures in the daytime, materially higher than those shown en route to Capetown. In fact, these high temperatures also occur in July. Yet cooling must be achieved as soon as possible after picking. For this reason alone it should be done at the packing house. Another reason is that the arrangement of the fruit for a maximum cooling rate is far easier at the packing house than in the railway cars.

Cooling the fruit is a quite different problem from just keeping it cool. The rate of cooling is affected materially by the difference in temperatures between that of the fruit and that of the air. An air temperature of 68°F, for instance, that would serve admirably in keeping the fruit cool would contribute only very slowly to lowering fruit temperature from 75°.

---

[3] Perry, R. L. Evaporative Cooling for Oranges. (personal notes) Dept. of Agr. Eng., University of California, Los Angeles.

Table 1. Mean hourly temperatures (F°), specified hours in September-October, Transvaal to Capetown (2).

Route stations	12 noon	2 p.m.	4 p.m.
Pietersburg	73°	76°	75°
Pretoria	73	77	77
Germiston	70	73	73
Bloemfontein	71	73	73
Victoria West	68	73	73
Beaufort West	69	73	74
Matroosburg	62	65	65
Capetown	64	66	64

Table 2. Daily maximum temperatures (F°), frequency distribution (%) in September-October, Transvaal to Capetown (2).

Route stations	Below 65°	65°-75°	75°-85°	85°-95°	95° plus
Pietersburg	5%	26%	43%	24%	1%
Johannesburg	8	26	46	9	0
Bloemfontein	9	30	46	14	-
Bethulie	10	29	45	15	-
Capetown	33	49	15	3	0

Table 3. Mean hourly temperatures (F°), specified hours in September-October, Transvaal to Capetown (2).

Route stations	9 p.m.	1 a.m.	4 a.m.	7 a.m.
Pietersburg	62°	56°	54°	57°
Pretoria	64	58	54	56
Germiston	61	55	53	54
Bloemfontein	58	53	49	53
Victoria West	57	48	44	44
Beaufort West	62	55	52	51
Matroosburg	52	46	45	46
Capetown	55	54	53	53

Table 4. Daily minimum temperatures (F°), frequency distribution (%) in September-October, Transvaal to Capetown (2).

Route stations	Below 37°	37°-47°	47°-57°	57° plus
Pietersburg	2%	27%	57%	14%
Johannesburg	4	30	54	12
Bloemfontein	15	36	40	9
Bethulie	19	41	31	9
Capetown		16	76	8

Table 5. Mean hourly relative humidities (%), specified hours in September-October, Transvaal to Capetown (2).

Route stations	12 noon	2 p.m.	5 p.m.
Pietersburg	40%	36%	38%
Middleburg		36	
Pretoria	37	32	30
Germiston	39	34	35
Kroonstad		36	
Bloemfontein	31	27	27
DeAar		27	
Victoria West	25	24	23
Beaufort West	34	28	27
Matroosburg	43	39	40

Table 6. Mean hourly relative humidities (%), specified hours in July, Transvaal to Capetown (2).

Route stations	12 noon	2 p.m.	5 p.m.
Pietersburg	39%	35%	38%
Middleburg		33	
Pretoria	36	31	29
Germiston	38	32	35
Kroonstad		31	
Bloemfontein	38	32	33
DeAar		34	
Victoria West	45	36	34
Beaufort West	42	36	35
Matroosburg	57	50	51

The first step in countering the heat is to protect the fruit from the sun and hot wind from the moment it is picked. Early morning picking is to be encouraged, if the fruit is dry. The use of canvas to shield the fruit picked into trailers was observed in this area and such protection is good. Immediate transport to the protection of the packing house is important.

At the packing house there are a number of measures that will help. Protection from the sun and hot wind is necessary. Maximum use of both night cooling techniques and of evaporative cooling must be developed.

Data for analyzing the prospects for cooling the fruit at the packing houses are limited but some indications can be given. The July data can be summarized by saying that night temperatures are low enough for good night cooling. In September-October, however, the prospects are variable. At Zebediela the mean of daily minimum temperatures is 56° F. At White River the mean monthly minimum is 55°. At Nelspruit the mean of hourly data is 66° at 9:00 p.m., 59° at 1:00 a.m., 57° at 4:00 and 60° at 7:00 (2). These specific temperatures are low enough, even for cooling fruit effectively, but one can infer that some nights are considerably warmer. It seems probable that almost all nights are cool enough for a few hours to hold fruit at an adequately low temperature, but it also seems certain that there will be a few nights when effective cooling rates will not be possible from simple night cooling, that is, to temperatures to or below 70°. Cooling down to at least 75° may be practical and as a first step in cooling could be very important.

Humidity levels are high enough to make evaporative cooling an uncertain factor. At Zebediela in September-October the relative humidity is above 50% on 31% of the days. The humidity is between 40 and 50% on 17% of the days[4]. Consequently, only half the time during these critical months can evaporative cooling be thought of as really effective. At Nelspruit the average relative humidity at 2:00 p.m. in September-October is 44%. However, even at that humidity level, 85° air can be lowered to 70° (Fig. 3). Properly used, this very low cost method of cooling can help to reduce the threat of black spot for at least an important proportion of the shipping days during these 2 months.

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[4] Data from African Realty Trust Ltd.

With these slight differences in air temperatures, moving the air among the containers may not be enough to cool the fruit; the air may have to be moved through the fruit (4). California packing houses that use evaporative cooling methods always use water cooled as much as possible. Ice often is introduced into the system on particularly hot days. The cost of these various devices is relatively low (3).

The map (Fig. 1) shows that Middleburg is near the packing houses at Nelspruit and White River and is on the route to Capetown. It is at a high altitude with cool nights, and relative humidity would be low. The temperatures and humidities at Johannesburg, Pretoria, and Germiston (Tables 3, 4, 5 and 6) are very favorable and they are only 200 miles distant. Even if the fruit is not quite down to 70° when shipped, proper handling of the railway cars may reduce temperatures sufficiently more.

Maximum use should be made of all methods of cooling the fruit. Careful study, design, and management are the only difficulties in the total program.

OTHER CONSIDERATIONS

The use of evaporative cooling and also of night cooling will raise the humidity around the fruit. Calavan indicates there is some evidence that higher humidity in itself would aid in the control of black spot. "In polyethylene bags containing a very moist atmosphere, the expression of black spot was delayed and reduced in frequency..."[5].

High relative humidity also is regarded as desirable for the maintenance of good texture and the prevention of shrinkage. Care must be used, of course, to prevent condensation of moisture on the fruit, which often accelerates the growth of molds. Generally, however, blue and green mold growth is materially slowed by the lowering of temperatures.

The proposed program involves important changes in packing houses and in the design and operation of railway cars. All these matters require a more extended analysis than is possible here.

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CALCIUM NITRATE FOR FIELD CONTROL OF TOMATO SOUTHERN BLIGHT IN SOUTH CAROLINA[1]

W. R. Sitterly[2]

Abstract

Granular calcium nitrate, applied as a side-dressing at the rate of 100 pounds per acre as soon as transplants are established, will adequately reduce and delay the appearance of Sclerotium rolfsii on tomato in South Carolina.

Granular calcium nitrate did not need soil incorporation to be effective where a cultural procedure of applying soil to the base of the plant as it matured was followed. In application, care was taken not to place granules in contact with tomato stem.

Losses on tomatoes from the southern blight organism (Sclerotium rolfsii Sacc.) in coastal South Carolina may be as high as 40% of a specific crop. The severity of the disease varies greatly from year to year and from field to field in the same year. Because of its widespread and sporadic occurrence and because it attacks plant parts in direct contact with the soil, it is most difficult to control effectively. To be effective, any practice must be sufficiently inexpensive so that a grower may use it every year as insurance against a disease outbreak.

The use of calcium nitrate is not new in agriculture, but most of the work with this material has been concerned with its use as a fertilizer. In a recent review paper, Harrison (1) noted that attempts have been made to control southern blight on specific crops by use of nitrogenous fertilizers, including calcium nitrate, since 1935. Most of the positive results obtained have been attributed to the effects of the nitrogen either upon the host or upon the microflora antagonistic to S. rolfsii.

Interest in the use of nitrogenous fertilizers to control southern blight was revived by Watkins, Mohr, and Young (3) in 1958 when they reported that the incidence of this disease on tomato was reduced and the appearance was delayed following an application of 150 to 200 pounds of calcium nitrate per acre as a side-dressing treatment. Young (4, 5, 6) and Mohr and Watkins (2) presented further data indicating the effectiveness of calcium nitrate. In greenhouse studies, Mohr and Watkins obtained evidence that reduced susceptibility of tomato plants to attack by S. rolfsii was due to stimulation of the meristematic activity of the phellogen by the increased quantities of available calcium.

The work reported here was initiated to evaluate the effectiveness of calcium nitrate side-dressings as a means of reducing losses from southern blight in tomato fields in South Carolina.

PROCEDURE

Calcium nitrate in granular form was tested for the control of southern blight on tomatoes over a 3-year period from 1959 to 1961. Other chemical compounds used in one or more years were calcium sulfate, basic slag, and the soil fungicide pentachloronitrobenzene (PCNB). The tests were conducted on a Nosbig fine sandy loam soil on the farm of the Clemson College Truck Experiment Station at Charleston, South Carolina.

A randomized block design with a minimum of five replicates was used in all tests. The individual plots were single, 20-foot rows of Marion tomatoes spaced 2 feet apart in the row. Both fall and spring tests were conducted.

All of the chemicals, except the PCNB and transplant solutions, were applied to the soil surface and were not incorporated into the soil. The PCNB was applied in a trench alongside the plants and covered, or was mixed with fertilizer, applied broadcast, and mixed into the soil before the land was bedded. Transplant solutions were poured around the base of the plants at a rate of 1 pint per plant.

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Table 1. Effect of specific chemicals and of methods and time of application on control of southern blight of tomato, Charleston, South Carolina.

Chemical	Rate (lb/acre)	Method of application	Time of application	Plants infected
1959				
PCNB[a] 40% dust	18	Broadcast	Preplant	5.0
PCNB 75 W	18	Transplant water	Transplant	7.9
PCNB 40% dust	18	Row	Transplant	12.5
Calcium nitrate	100	Side-dress	Transplant	0.0
Control				10.0
1960				
PCNB 40% dust	18	Broadcast	Preplant	47.5
PCNB 75 W	18	Transplant water	Transplant	28.0
Calcium nitrate	100	Side-dress	Transplant	22.0
Calcium nitrate	100	Side-dress in alley[b]	Transplant	35.0
Calcium nitrate	100	Side-dress	Last cultivation	40.0
Calcium nitrate	100	Side-dress in alley[b]	Last cultivation	47.0
Calcium sulfate	200	Side-dress	Last cultivation	65.0
Basic slag	200	Side-dress	Last cultivation	45.0
Control				65.0
1961				
PCNB 75 W	18	Transplant water	Transplant	10.0
Calcium nitrate	450	Transplant water	Transplant	(80% killed by the chemical)
Calcium	100	Side-dress	Transplant	10.0
Control				33.0

[a] Pentachloronitrobenzene (Terraclor).

[b] Side-dress applications for these treatments were applied 15 to 18 inches from the plants in a small furrow in the alley. All other side-dress applications were located 4 to 6 inches from the base of the tomato transplants.

The number of infected plants was determined as a measure of disease severity. A plant was considered to be infected if the stem or one or more branches were girdled by *Sclerotium rolfsii*.

RESULTS

The results of the initial experiment conducted in 1959 indicated definitely that further trials were warranted, when no infected plants occurred in the calcium nitrate plots in contrast to the 10% infected plants in the control plots (Table 1). PCNB was not so effective as calcium nitrate.

1960: Tests were expanded in 1960 to determine if the source of calcuim, purported (2) to be the key element in development of a mechanical barrier, was important. In these tests granular calcium nitrate proved more effective than calcium sulfate or basic slag (Table 1). Most effective control with calcium nitrate was obtained when it was applied as a side-dress application on the edge of the bed alongside the young plants immediately following transplanting. Less effective control was obtained when the chemical was applied 15 to 18 inches away from the plants or when the application was delayed until the time of the last close cultivation, which is when side-dress applications of additional nitrogen fertilizer are normally made to tomatoes in South Carolina. PCNB, applied at a rate of approximately 18 pounds per acre in the transplant water, proved almost as effective as the calcium nitrate. It was relatively less effective, however, when applied at the same rate as a broadcast application with the fertilizer. Calcium nitrate did not need incorporation into the soil if a cultural procedure of applying soil to the base of the plants as they matured was followed.

1961: Although yields were low because of adverse environmental conditions during fruit setting, the 1961 data confirmed results obtained in 1959 and 1960 (Table 1). When calcium nitrate was dissolved in transplanting water, at the high rate of 100 pounds in 100 gallons, it was toxic to bare-rooted tomato transplants. Although the control obtained from usage of PCNB was equal to that obtained from usage of calcium nitrate, the yield was significantly lower than on the calcium nitrate treated plots. The yields were 10,200 pounds, 13,800 pounds, and 10,000 pounds per acre, respectively, for PCNB, calcium nitrate, and control plots.

DISCUSSION

The most effective control of southern blight on tomatoes was obtained with an application of 100 pounds of granular calcium nitrate per acre, applied in a band adjacent to the young plants as soon as the plants became established after transplanting. Based on the theory proposed by Mohr and Watkins (2), early application would be essential to allow the plants to absorb and utilize the available calcium for periderm formation before environmental conditions are conducive to the development of S. rolfsii. It would also be essential that the chemical be placed close enough to be picked up quickly by the limited root systems of the newly transplanted seedlings. Thus, the theory tends to explain the effective control obtained in South Carolina and the erratic control obtained by Young (4, 5, 6) in Texas, when the chemical was applied 6 weeks after the plants were transplanted. Young usually obtained adequate control with calcium nitrate, but reported it to be less effective than PCNB. The greater effectiveness of calcium nitrate in South Carolina probably resulted from its availability to the young plant early in the season.

Calcium nitrate is a source of readily-available nitrogen for plant growth. Thus, in order to avoid use of excessive nitrogen, growers will have to reduce the amount of nitrogen in the initial fertilizer application or in the side-dress application normally made at the time of the last cultivation.

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PEACH MOSAIC VIRUS IN HORTICULTURAL PLUM VARIETIES[1]

T. S. Pine[2] and L. C. Cochran[3]

Abstract

Forty-two of 43 horticultural plum varieties tested were found to be hosts for at least one of the two strains of the peach mosaic virus tested. No correlation could be detected between heredity and varietal reactions. Disease expression was more evident in trees on commercial rootstocks than in own-rooted trees. Plum varieties reacted differentially to infection with two strains of the virus.

INTRODUCTION

Horticultural plum varieties are rarely found naturally infected with the peach mosaic virus (2), but they are susceptible to infection when artificially inoculated with the virus (1). Foliar symptomatology seems to vary, depending upon the virus source. Because commercial rootstocks (marianna, Myrobalan, peach) are all highly susceptible to the virus, it was assumed that the rootstocks might directly affect the expression of disease symptoms by scion varieties. The investigations reported here were formulated to test this assumption and to explore variations in disease expression caused by different virus strains.

MATERIALS AND METHODS

During 1942-1954, 26 varieties of plums (Prunus cerasifera, P. domestica, P. insititia, and P. salicina) were grown on commercial rootstocks at Riverside and inoculated, by means of bud grafts, with peach mosaic virus from two sources. Culture PM-1, which was obtained from peach, produced severe disease symptoms in peach but not in Royal apricot. PM-2, originally from a diseased apricot tree, produced striking symptoms in both peach and Royal apricot. Two or more trees of each plum variety were inoculated with budwood infected with each virus strain. The year after inoculation budwood was taken from the plum test trees and placed in healthy peach trees. The results of the inoculations and the subsequent back indexings to peach are shown in Table 1.

Between 1956 and 1961, 37 varieties of plums were successfully grown from hardwood cuttings and inoculated with the same virus cultures used in the earlier work. Two trees of each variety received budwood from each virus source. Back indexing to healthy peach trees was carried out in the manner mentioned above. When symptoms were inconclusive in the index trees, a second index was used. Unfortunately it was not possible to produce own-rooted hardwood cuttings of all the varieties used in the 1942-1954 investigations. The results of these inoculations and indexes are also shown in Table 1.

RESULTS AND DISCUSSION

Of the 43 horticultural plum varieties used in both phases of this investigation, 42 were found to be hosts of one or both virus strains. President was immune from PM-1 and PM-2 when rooted on commercial stocks but susceptible when self-rooted. Only Mariposa can be considered truly immune, on the basis of its reaction in the more sensitive, own-rooted category. No explanation is offered for this phenomenon since Satsuma, which most closely resembles Mariposa, is highly susceptible.

Differential immunity from either PM-1 or PM-2 was evident in four varieties growing on rootstocks and six own-rooted varieties. In the first group Late Santa Rosa, President, Santa Rosa, and Standard were immune from PM-1, but only President showed immunity from PM-2. Because of the general immunity of President, evidently PM-2 was the more aggressive of the two virus strains when plums were grown on commercial stocks. In the own-rooted group

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the California Agricultural Experiment Station, Riverside.

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Table 1. Reactions of plum varieties grown on commercial rootstocks and on their own roots to inoculation with two peach mosaic virus strains.

	Symptoms when on rootstocks		Virus recovered in peach		Symptoms when on own roots		Virus recovered in peach	
Variety	PM-1	PM-2	PM-1	PM-2	PM-1	PM-2	PM-1	PM-2
Abundance	+	+	+	+	+	+	+	+
Apex					+	+	+	+
Beauty	+	+	+	+	0	0	+	+
Becky Smith	+	+	+	+	+	+	+	+
Black Prince	0[a]	0[a]	+	+	+	0	+	+
Burbank	+	+	+	+	+	0	+	+
Burmosa					+	+	+	+
Climax					0	+	+	+
Damson	+	+	+	+	+	+	+	+
Duarte	0[a]	0[a]	+	+	+	+	+	+
Elephant Heart					+	+	+	+
Emelie	+	+	+	+	+	+	+	+
Flaming Delicious	+	+	+	+	0	0	+	+
Formosa					+	+	+	+
French	+	+	+	+				
Gaviota					0	+	+	+
Giant	+	+	+	+	+	+	+	+
Green Gage	+	+	+	+	0	0	+	+
Hollywood					0	0	+	+
Hungarian	+	0[a]	+	+	0	0	+	0
Italian	+	+	+	+				
Kelsey	+		+		+	+	+	+
Laroda					0	0	+	+
Late Santa Rosa	0	0[a]	0	+	+	+	+	+
Mariposa					0	0	0	0
Methley					+	+	+	+
Myrobalan	+	+	+	+	+	+	+	+
Nubiana					0	0	+	+
Padre					+	+	+	+
President	0	0	0	0	0	0	+	+
Queen Ann					0	0	0	+
Redheart					0	0	0	+
Red June	+	+	+	+				
Redroy					0	0	+	0
Santa Rosa	0	0[a]	0	+	+	+	+	+
Satsuma	+	+	+	+	+	+	+	+
Shiro	0	+	+	+				
Shropshire					+	0	+	+
Standard	0	0[a]	0	+				
Sugar	0	+	+	+				
Tragedy	0[a]	+	+	+	+	+	+	+
Wickson	0	0[a]	+	+	0		0	
Yakima					0	0	+	+

[a] Symptoms appeared in the rootstocks.

Mariposa, Queen Ann, Redheart, and Wickson showed immunity from PM-1, and Hungarian, Mariposa, and Redroy failed to retain PM-2. Considering the general immunity of Mariposa in this group, the PM-2 strain was more aggressive. These observations confirm earlier work which suggested that plum varieties reacted differentially to infection with various strains of the peach mosaic virus. The fact that PM-2 strain, which originated in apricot, was more aggressive in plums than the PM-1 strain, from peach, substantiates other work with peaches and apricots which has shown PM-2 capable of producing more severe disease symptoms in a greater number of *Prunus* varieties than PM-1 (unpublished data).

The number of varieties in which one or both virus strains remained latent was much greater in the own-rooted than in the topworked group. The data clearly indicate that the presence of a susceptible rootstock in the system leads to increased disease expression. Nine varieties on commercial roots carried PM-1 or PM-2 in latent form; 17 varieties reacted similarly on their own root systems. There was no apparent correlation between latency of either virus strain and the root system concerned. Both strains were latent in Duarte on commercial stocks but produced symptoms on self-rooted trees. Beauty, Flaming Delicious, and other varieties showed symptoms of both virus strains when grown on rootstocks but carried the cultures in latent form when on their own roots. However, in two instances the presence of a rootstock resulted in immunity from the PM-1 strain. Late Santa Rosa and Santa Rosa on commercial roots were immune from this strain but susceptible when self-rooted.

No correlation could be found between heredity and the degree of susceptibility, latency or immunity exhibited by the varieties under study. Varieties of the principal species used (*P. domestica* and *P. salicina*) fell into all three categories of response to inoculation with the virus strains. Apparently, the observed disease reactions were related to the particular variety without regard to root system or species.

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STEM AND CROWN NECROSIS OF COASTAL BERMUDA GRASS CAUSED BY HELMINTHOSPORIUM SPICIFERUM[1]

Robert T. Gudauskas[2]

Abstract

Helminthosporium spiciferum (Bain.) Nicot was found to cause a stem and crown necrosis of Coastal Bermuda grass, Cynodon dactylon. Infection occurred only when an aqueous spore and mycelial suspension was injected directly into stems and crowns of healthy plants. Relative importance of the disease in Alabama is undetermined.

INTRODUCTION AND LITERATURE REVIEW

During the summer of 1961, Coastal Bermuda grass, Cynodon dactylon, in western Alabama was affected by what appeared to be a crown and root rot disease. Plants were dead or dying and manifested various stages of decay in stem, crown, and root tissues. The disease was restricted to a small area within one field and was associated with a heavy infestation by the meadow spittlebug, Philaenus leucophthalmus. Although spittlebug damage on the aerial portions of plants was apparent throughout the entire field, only those plants within the restricted area mentioned above had necrotic stems, crowns, and roots.

Isolations made from the diseased tissues consistently yielded Helminthosporium spiciferum (Bain.) Nicot; synonyms are Brachycladium spiciferum Bain. and Curvularia spicifera (Bain.) Boedijn[3]. The fungus has been reported as a saprophyte commonly associated with root and crown tissues of many graminaceous plants, excluding Coastal Bermuda (2, 4, 5, 10, 12, 13, 14). Luttrell (7) isolated the fungus from seed heads of diseased smooth brome, and others have isolated it from seed of oats (3), rice (11), and crimson clover (6). Helminthosporium tetramera McK., which is listed as an identical species by some workers (2, 4, 5, 13), is capable of causing foot-rot of wheat (2, 8, 9) but is regarded as non- to weakly pathogenic on most cereals (1, 5, 9, 13).

Since this was apparently the first report of H. spiciferum associated with a disease condition in Coastal Bermuda grass, investigations were undertaken to determine if the fungus were pathogenic. The results of the tests are reported.

MATERIALS AND METHODS

The inoculum used in all tests was a suspension of spores and mycelia in sterile, deionized water made from 10-day-old cultures of H. spiciferum growing on potato-dextrose agar. Test materials were healthy Coastal Bermuda plants grown to a height of 8 to 10 inches from sprigs in sterilized soil in pots and flats. All tests were conducted in the greenhouse, and plants were subjected to prevailing conditions of watering, lighting, and fertilization.

Three different inoculation procedures were employed: 1) The aerial portions of test plants were sprayed (hand atomizer) with inoculum, 2) the soil in which test plants were growing was thoroughly infested by drenching with inoculum, or 3) the main stems or crowns of test plants were injected with inoculum by means of a hypodermic syringe fitted with a No. 26 hollow-point needle. Stems were injected at various points between the soil line and the first leaf. Wounds were covered with vaseline.

Controls were plants treated with sterile, deionized water applied by the spray, infestation, or injection method. Plants were held in a moist chamber for 48 hours following inoculation and then returned to greenhouse benches.

RESULTS

Of the three methods used, only the direct injection of inoculum into test plants resulted in noticeable infection. All 12 plants inoculated by a crown injection became infected, while 6 of 10 plants became infected following stem injection. In contrast, none of the 18 plants

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[1] Published with the approval of the Director of the Alabama Agricultural Experiment Station.

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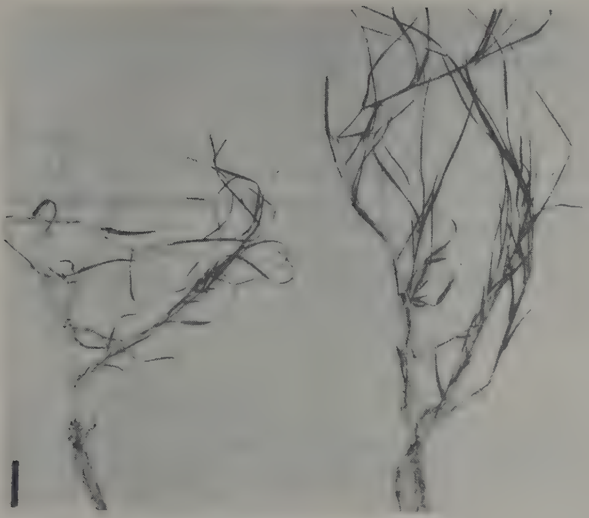
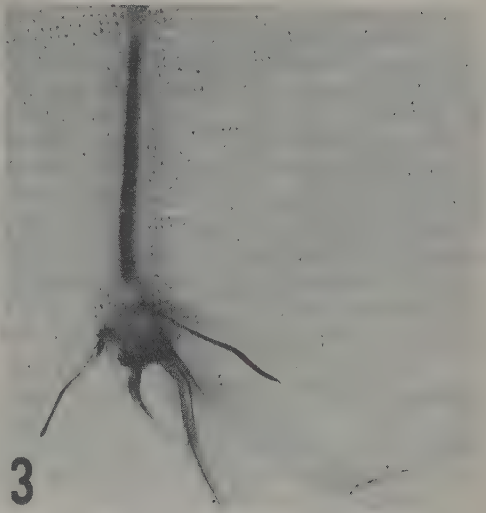
[3] The author expresses his appreciation to Dr. E. S. Luttrell, Georgia Experiment Station, Experiment, Georgia for identification and synonyms of the fungus.





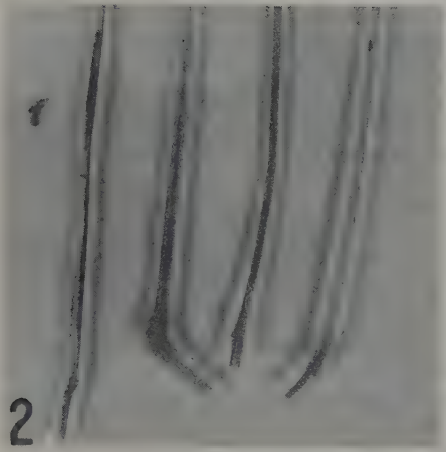


FIGURE 1. Left -- Coastal Bermuda grass on 30th day following injection of inoculum into main stem. Right -- control.

FIGURE 2. Coastal stems split lengthwise to show development of necrosis following inoculation by stem injection. Note extent of necrosis, blackened areas, as compared with control on far right.

FIGURE 3. Lower portion of a Coastal plant split lengthwise to show development of necrosis by the 30th day following crown injection. Note spread of rot, grey to black in color, into stem and roots from the infected crown.

inoculated by spraying or soil infestation became infected.

In repeated tests using stem injection, 10 of 15 and 24 of 25 plants inoculated became infected.

Helminthosporium spiciferum was recovered in pure culture from diseased tissue of all infected plants, whether inoculated by stem or crown injection.

Symptoms on Experimentally Infected Plants: The following observations were made on the 30th day after inoculation by stem or crown injection.

External. Infected plants were stunted, spindly in appearance, and tended to fall over although stems did not break (Fig. 1). Dead and dying leaves, which were brown or chlorotic, were apparent on infected plants, particularly in those areas adjacent to injection points.

Stem and crown lesions were dark purple to black in color, while those that occasionally developed on controls were brown. Lesions generally did not increase in size superficially beyond that of the original wound, although internal discolorations extending beyond the lesion areas were apparent.

Roots of plants inoculated in the crown were rotted and brown in color.

Internal. The symptom observed in infected tissues was a dark brown to black, moist rot. In stem-inoculated plants, infection was usually, but not always, confined to the pith between nodes (Fig. 2). Necrotic vascular tissues that appeared as brown streaks extending internodally beyond the main stem necrosis were occasionally observed. Necrosis that had originated near the soil line in stems but later spread into crowns also occurred.

Inoculated crowns were completely rotted. Necrosis that had spread up into stems and downward into roots from an inoculated crown was frequently observed (Fig. 3).

CONCLUSION AND DISCUSSION

The data obtained through a fulfillment of Koch's Postulates show that Helminthosporium spiciferum was pathogenic on Coastal Bermuda grass. The infection of stem and crown tissues by the fungus resulted in necrosis leading to marked weakening and eventual death of affected plants.

The importance of the disease on Coastal Bermuda grass in Alabama is unknown. Although observed in only one restricted area during 1961, the disease could easily have been overlooked or attributed to other factors causing similar symptoms. It is possible that this disease was a contributing factor to the die-back and degeneration that has occurred in some Coastal Bermuda fields in Alabama during recent years.

The original discovery of the disease in association with a spittlebug infestation indicates a relationship worthy of further investigation since H. spiciferum is apparently incapable of penetration, requiring a wound for access and subsequent infection of Coastal Bermuda plants.

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ROOT KNOT AND THE SEEDLING DISEASE COMPLEX OF COTTON[1]

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Summary

Greenhouse tests with a nematocide and fungicide on naturally infested soil indicate an interaction between *Meloidogyne incognita* and seedling disease and root rot fungi. Inoculation of sterile soil with *M. incognita* plus *Rhizoctonia solani* and *M. incognita* plus *Thielaviopsis basicola* resulted in more severe disease symptom expression and reduction of growth than when inoculated with either pathogen alone.

INTRODUCTION

Numerous reports in the literature demonstrate that root-knot nematodes, *Meloidogyne* spp., influence the severity of Fusarium wilt of cotton (2, 4, 7). Holdeman and Graham (1) demonstrated that the sting nematode, *Belonolaimus gracilis* Steiner, facilitates the development of Fusarium wilt in cotton plants. The root-knot nematode has been shown to increase the severity of other fungal (5, 8) and bacterial wilt diseases (3).

Rhizoctonia solani Kuehn and *Thielaviopsis basicola* (Berk. & Br.) Ferr. are responsible for the greatest losses due to seedling disease of cotton in New Mexico. Reynolds and Hanson (6) demonstrated that an increase in the severity of Rhizoctonia disease was associated with an increase in the incidence of root-knot nematodes and with a corresponding decrease in plant size and survival.

This paper is a summary of experiments on the interaction of root-knot nematodes, *Meloidogyne incognita* (Kofoid & White) Chitwood, and seedling diseases of cotton caused by *R. solani* and *T. basicola*.

MATERIALS AND METHODS

Soil from an area known to be severely infested with *Meloidogyne incognita* and various root rotting and seedling disease fungi was placed in 6-inch clay pots and treated as follows: 1) no treatment, 2) 1,2-dibromo-3-chloropropane (Nemagon EC-2), 3) pentachloronitrobenzene (Terraclor) and 4) a combination of Nemagon and Terraclor. The chemicals were applied as drenches at rates commonly used in the field. Each treatment was replicated five times. Each pot was planted with five seeds of acid delinted, untreated, Acala 1517C seed and watered at regular intervals. Data were taken after 4 weeks.

In a second experiment steam-sterilized soil was placed in 6-inch pots, replicated five times, and randomized. The treatments were as follows: 1) check-sterile water, 2) *Rhizoctonia solani*, 3) *Meloidogyne incognita*, and 4) *R. solani* and *M. incognita* added in combination. The fungus inoculum was obtained by emptying a culture of the fungus just covering a Petri dish of potato-carrot-dextrose agar into a blender with 100 ml of sterile water and blended for 1 minute. The content of one Petri dish was added to appropriate pots. The nematodes were hatched from egg masses grown on tomato roots, rinsed through three changes of sterile water, and aliquots containing approximately 3000 larvae added to appropriate pots by pipetting. Five seeds of 1517C cotton were planted in each pot and adequate moisture was maintained by adding distilled water as needed. Data were taken after 21 days.

A third experiment was identical to the inoculation study above with the exception of the addition of *T. basicola* instead of *R. solani*. Data were taken after 21 days.

In a fourth experiment, field tests were conducted on a field known to be infested with nematodes and with a cropping history conducive to having a seedling disease problem. The following treatments were replicated four times: 1) untreated check, 2) Terraclor applied as an in-furrow spray at planting, 3 pounds per acre, 3) Nemagon EC-2 applied at the rate of 8.6 pounds technical per acre as an in-furrow spray at planting, 4) Nemagon S-1 pre-

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Table 1. Effect of a nematocide and a fungicide on the amount of root knot, seedling disease, and plant weights in the greenhouse.

Treatment	Amount of root knot[a]	Amount of seedling disease[b]	Dry weight of plants (in grams)[c]
Check	2.5	5.0	8.2
Nemagon EC-2, 1/2 gal. Tech. /A	1.0	3.0	17.1
Terraclor WP, 3 lb/A	2.6	2.0	18.0
Nemagon, 1/2 gal. Tech. /A + Terraclor 3 lb/A	1.0	1.6	21.0
LSD 5%	.6	.8	9.1
LSD 1%	1.1	1.5	11.0

[a] Average root-knot index 0 = no galling, 5 = galling severe.

[b] Average seedling-disease index 0 = no seedling-disease symptoms, 5 = roots blackened, non-functional.

[c] Average dry weight per plant, dried at 140° F for 48 hours.

Table 2. Influence of root-knot nematodes on cotton seedling disease induced by *Rhizoctonia solani* in greenhouse cultures.

Treatment	Nematode index[a]	Seedling-disease index[b]	Dry plant weight (in grams)[c]
Check	0	2.1	19.8
Rhizoctonia solani	0	4.2	12.6
Meloidogyne incognita	3.6	2.5	10.7
Rhizoctonia + *Meloidogyne*	3.5	5.0	9.3
LSD 5%		.8	4.9
LSD 1%		1.6	8.3

[a] Average root-knot index 0 = no galling, 5 = galling severe.

[b] Average seedling-disease index 0 = no seedling-disease symptoms, 5 = roots blackened, non-functional.

[c] Average dry weight per plant, dried at 140° F for 48 hours.

Table 3. Influence of root-knot nematodes on cotton seedling disease induced by *Thielaviopsis basicola* in greenhouse cultures.

Treatment	Nematode index[a]	Seedling-disease index[b]	Dry plant weight (in grams)[c]
Check	0	1.0	21.4
Thielaviopsis basicola	0	3.6	16.3
Meloidogyne incognita	3.0	1.6	14.2
Thielaviopsis + *Meloidogyne*	3.4	4.3	11.1
LSD 5%		.7	8.9
LSD 1%		1.5	12.5

[a] Average root-knot index 0 = no galling, 5 = galling severe.

[b] Average seedling-disease index 0 = no seedling-disease symptoms, 5 = roots blackened, non-functional.

[c] Average dry weight per plant, dried at 140° F for 48 hours.

Table 4. Field response of Pima S-1 cotton to Nemagon S-1 and Terraclor expressed as yield and root-knot index.

Treatment	Yield/acre seed cotton	Root-knot index[a]
Terraclor 3 lb/A + Nemagon EC-2, 1/2 gal. Tech. /A in-furrow spray	1076	3.15
Terraclor 3 lb/A in-furrow spray	1023	2.15
Nemagon EC-2, 1/2 gal. /A in-furrow spray	1071	1.75
Nemagon S-1, 1/2 gal. /A preplant chisel application	1591	1.25
Untreated check	1057	2.05
Nemagon S-1 preplant chisel + Terraclor in-furrow spray	1497	1.45
Water check, in-furrow spray	1016	2.00
LSD 5%	185	.5
LSD 1%	260	1.0

[a]Average root-knot index 0 = no galling, 5 = galling severe.

plant chisel application at the rate of 8.6 pounds technical per acre, 5) chisel application of Nemagon S-1, 1/2 gallon per acre followed by Terraclor 3 pounds per acre as an in-furrow spray at planting, 6) Nemagon EC-2, 1/2 gallon per acre and Terraclor 3 pounds per acre applied as an in-furrow spray at planting and 7) in-furrow spray water check. The field was planted with acid-delinted Pima S-1 at the rate of 20 pounds seed per acre. Stand counts and yield weights were recorded.

RESULTS

The growth response of cotton from the application of Nemagon and Terraclor on naturally infested soil in the greenhouse is given in Table 1. Nemagon EC-2 applied as a drench reduced nematode damage expressed as reduced galling. The nematocide reduced the root rot and seedling-disease symptoms but not to the degree as did the fungicide or the two materials in combination. All treatments resulted in a significant increase in plant growth when compared with the untreated check. The difference became more marked with increased time of pathogen exposure ending with death of the plants. There appeared to be an additive effect when the two chemicals were used in combination, as indicated by the extreme increase in plant weight compared with the increase from either of the two materials singly.

In the second experiment involving the addition of the R. solani and M. incognita to sterile soil, a low level of Rhizoctonia contamination was found in all pots. The addition of nematodes alone did not materially affect the severity of seedling disease expression (Table 2).

Plants inoculated with R. solani plus M. incognita were black, shriveled, and very few survived. When the two measures of plant damage, nematode index and seedling-disease index, were totaled for each treatment, the figures were roughly an inverse proportion to the dry plant weight.

Greenhouse studies using T. basicola as the fungus pathogen gave the same general response as Rhizoctonia (Table 3). Fungal contamination, primarily R. solani, tended to mask the response, but the overall pattern of black root rot symptoms was present. There was a significant interaction between T. basicola and M. incognita at the 5% level of error. The addition of nematodes reduced the dry plant weight by 1/3 when compared with the check, but the effect was not significantly different.

In experiment four there were no significant differences between stand counts in the field evaluation studies. Chisel applications of Nemagon S-1 resulted in an increase of 534 pounds per acre of seed cotton over the check, and the Nemagon S-1 followed by Terraclor resulted in an increased yield of 440 pounds per acre both of which were highly significant (Table 4). Nemagon EC-2 and Terraclor gave no significant economic return when applied singly or in combination.

DISCUSSION AND CONCLUSIONS

In the greenhouse test with naturally infested soil, treatments with a nematocide and fungicide indicates the presence of an interaction between the root-knot nematode, *M. incognita,* and the seedling disease and root rot fungi. Further studies with inoculated soil demonstrated an interaction between *Meloidogyne incognita*, *Thielaviopsis basicola*, and *Rhizoctonia solani* and substantiated the findings of others (6) concerning the action of nematodes in the overall seedling-disease complex. The mechanism whereby the interaction was realized was not a part of this preliminary study, but three possible explanations are offered: 1) the nematode parasites served to debilitate the plant (6) and make it more susceptible to attack by the fungi; 2) mechanical injury which provides portals of entry for strains of the fungus otherwise not capable of penetration and establishment; and 3) combinations of the aforementioned items.

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TRANSMISSION OF SOME GLADIOLUS VIRUSES ON TOOLS IN HARVESTING FLOWERS AND CORMS

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— cf-60. [illegible]

The tobacco ringspot virus was reported in 1960 (1) to be transmissible both by harvesting flowers and by cutting tops preparatory to harvesting of gladiolus corms. Similar tests were conducted in 1960 and 1961 to determine whether other viruses infecting gladiolus could be transmitted in like manner. In each test a virus-infected plant was cut immediately before each healthy test plant. Flower stalks were cut with a sharp knife and tops with shears. Results were determined in the following year by indexing the exposed gladioli on suitable test species (Table 1).

The ease of transmission of bean yellow mosaic corresponds to its prevalence in gladiolus, but it is transmitted also by aphids (4). The cowpea strain of this virus, also common in gladiolus (2), appears chiefly dependent on vector activity. Cucumber mosaic virus, slow-moving in gladiolus, became established after flower cutting (2 months before harvest) but not in the shorter interval (2 weeks) after cutting tops. Tomato ringspot virus, which failed to move by either method of contamination, seems dependent upon nematode activity (3).

Table 1. Virus transmission on tools in harvest of gladiolus.

Virus	Transmission by contamination in			
	cutting flowers		cutting tops	
	number	%	number	%
Tobacco ringspot	1/57	1.8	5/175	2.9
Tomato ringspot	0/26	0	0/40	0
Cucumber mosaic	4/46	8.7	0/109	0
Bean yellow mosaic	9/42	21.4	1/28	3.6
Cowpea strain of bean yellow mosaic	1/16	6.3	0/40	0

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PHYTOPHTHORA CROWN ROT OF PETUNIA[1]

Douglas J. Phillips and Ralph Baker[2]

Summary

A serious disease of petunia caused by Phytophthora parasitica has been found in greenhouses and home gardens near Denver, Colorado. Symptoms include a black discoloration and dry rot of the crown and lateral branches near the ground line, a general wilt, and eventual death of the plant. Control under greenhouse conditions was achieved by using steamed soil, flats and pots. Drenches or soil mixes using Dexon were also effective.

Since 1958 considerable losses of petunias (Petunia hybrida) have occurred in greenhouse bedding operations and in home gardens in the vicinity of Denver, Colorado. Phytophthora parasitica Dast.[3] was isolated consistently from diseased tissue. Inoculation and reisolation according to Koch's postulates confirmed pathogenicity.

Species of Phytophthora pathogenic to petunia have been reported by Dasteur in India (1), Pethybridge and Lafferty in Ireland (5), Moore in England (4) and Wiehe in Nyasaland (6). More recently, Haasis (2) has reported an isolate highly virulent to petunia.

SYMPTOMS

Petunias in infested soil developed symptoms after 1 to 4 weeks. When natural soil infestation occurred in greenhouses, infections developed typically in one area of a flat. Thereafter the rest of the plants in the flat developed symptoms rapidly. In home gardens plants generally became unthrifty soon after transplanting. In experiments conducted in the greenhouse the average incubation period was 2 weeks.

Symptoms included a black discoloration and dry rot of the crown and lateral branches near the soil line (Figs. 1, 2). This was accompanied by wilting and eventual death. The organism has been isolated from all portions of the stems and leaves.

Extensive experiments to determine the host range of the pathogen have not been conducted. Attempts to infect other plants reported to be hosts of P. parasitica, such as tobacco, pepper, and tomato, have been unsuccessful.

CONTROL

To test for the possibility of seed transmission, the seed of several varieties of petunia, including White Magic, Chiffon, and Comanchee, were surface-sterilized and plated on cornmeal agar. In all cases Phytophthora was not isolated. Other seeds of the same varieties were washed in running tap water and plated on pea straw natural medium (3). Several surface contaminants grew on the agar, but again Phytophthora was not detected. In repeated experiments, described later, non-inoculated controls in steamed soil never developed symptoms. Thus seed transmission would not appear to be a factor in dispersal.

In preliminary experiments, drenches and soil treatments with fungicides were attempted for control. Captan, O,O,O-trimethyl phosphorothioate (CH_3O) PS (Shell experimental compound SD-4741), cyano(methylmercuri) guanidine (Panogen), and p-dimethylaminobenzenediazo sodium sulfonate (Dexon) were effective.

Dexon was selected for further tests because of its effectiveness and probable availability for use on ornamentals. Steamed soils were infested with the organism or naturally infested soils were obtained. Repeated experiments demonstrated that Dexon was effective in control. A single representative experiment using naturally infested soil is described below.

Soil was collected from a home garden in Denver where extensive losses due to P. parasitica had occurred. Dexon was thoroughly mixed into the soil at rates of 5, 10, and 20 g active

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[3] Identification kindly made by Drs. D. C. Erwin and J. T. Barrett.

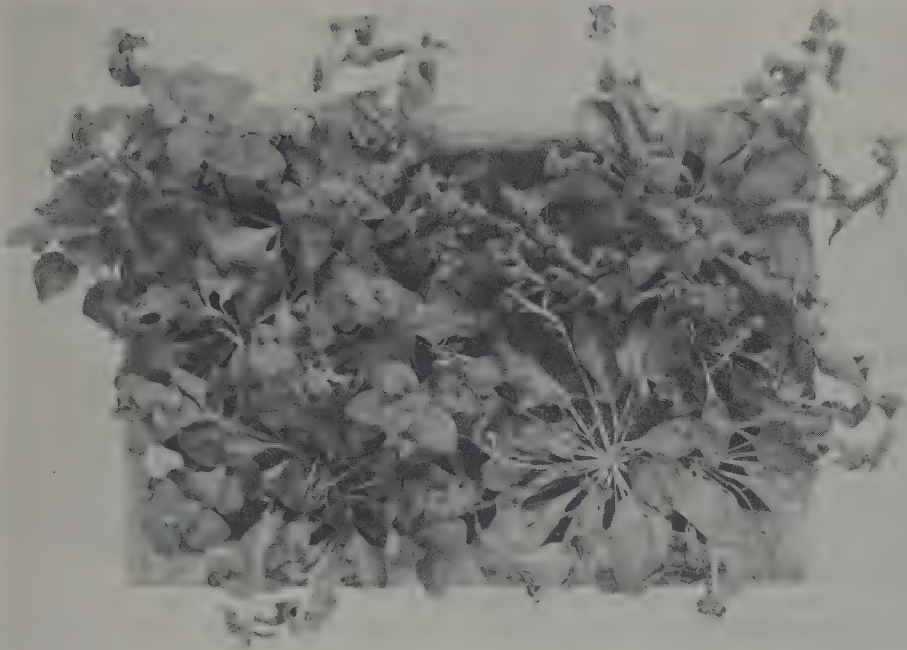

FIGURE 1. Symptoms of Phytophthora crown rot on petunias growing in a flat.

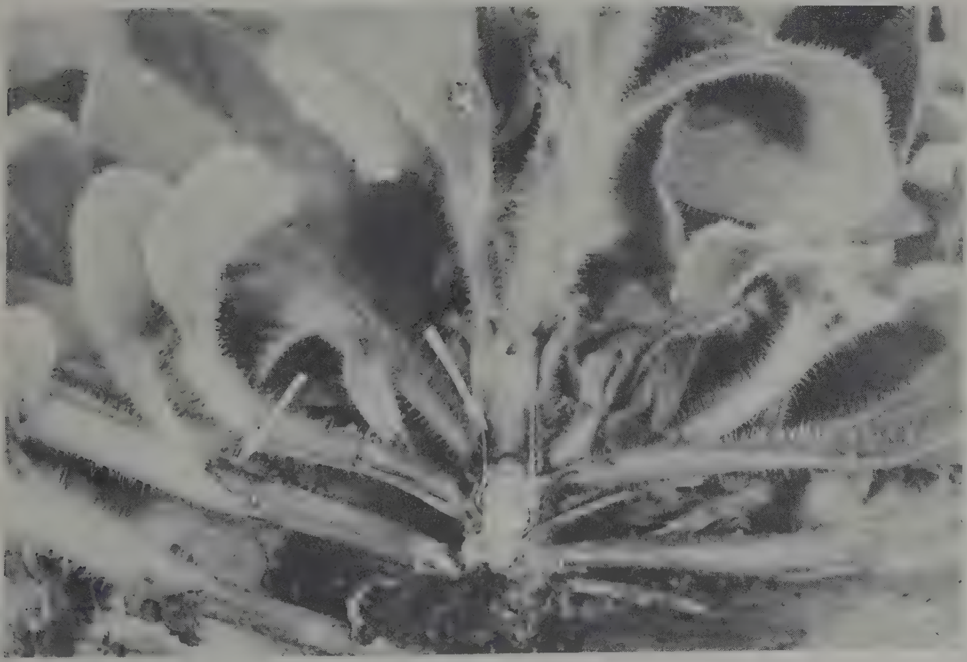

FIGURE 2. Section of crown split longitudinally. Arrows show black discoloration of crown and lateral branches.

per cubic foot of soil and placed into cardboard flats. In two additional treatments Dexon was applied to the soil weekly as a drench at 70 and 140 ppm (w/v) active. Petunias 45 days old were transplanted in each flat, eight plants in each. Each chemical treatment, the steam treated soil, and the non-treated controls contained four replications. Numbers of diseased plants were recorded each week for 4 weeks (Table 1).

Table 1. Control of Phytophthora crown rot of petunia with Dexon[a].

Rate	Method of application	% loss
5 g/ft^3 of soil	soil mix	34
10 g/ft^3 of soil	soil mix	12
20 g/ft^3 of soil	soil mix	7
70 ppm	weekly drench	12
140 ppm	weekly drench	3
Steamed soil		0
Non-treated soil		80

[a] Each treatment contained 32 plants in four replications.

All treatments significantly reduced the incidence of disease. Best control was achieved in steamed soil. The soil mix and the drench at the highest rates were effective. Some stunting of growth was observed in the former.

These results indicate that control may be achieved rather easily under greenhouse conditions by use of steamed soils and raised benches. If facilities for steaming or fumigation are not available, applications of Dexon should be practical. In preliminary experiments in home gardens, Dexon has not given as good control. Additional experiments are underway on this phase.

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EFFECTIVENESS OF DYRENE IN CONTROLLING STEMPHYLIUM LEAF SPOT OF BIRDSFOOT TREFOIL[1]

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Summary

Stemphylium leaf spot (Stemphylium loti) causes extensive defoliation of birdsfoot trefoil (Lotus corniculatus) in the southeastern United States. Loss of leaves ranged from 25 to 66% of the total leaves initiated. In preliminary tests with various fungicides and antibiotics, 2,4-dichloro-6-(o-chloroanilino)-s-triazine (Dyrene) was the most effective in suppressing Stemphylium leaf spot of trefoil. In 1959, 1960, and 1961 Dyrene (50% wettable powder) was applied to 6- x 7-foot plots of trefoil at weekly, biweekly and triweekly intervals and at 3, 4, 5, and 6 pounds of active chemical per acre for each application interval. The leaves initiated and the leaves infected and defoliated by S. loti were counted on 25 plants at random for each treatment at each harvest date. Each concentration of Dyrene at each of the application intervals reduced forage loss caused by S. loti defoliation. Application of 6 pounds/acre of Dyrene at weekly intervals increased total annual average forage yield by 2431 pounds/acre over untreated plots. Also, the numbers of leaves infected and defoliated, respectively, were 3.0 and 3.5 times greater in untreated plots than in plots with the most effective treatment (6 lb/acre, 7-day interval).

INTRODUCTION

Birdsfoot trefoil (Lotus corniculatus) is a long-lived, deep rooted perennial legume in areas where it is adapted. In the southeastern United States, however, trefoil is severely attacked by diseases, one of which is Stemphylium leaf spot caused by Stemphylium loti Graham. The most active stage of the disease occurs during May and June and again in mid-fall. In the spring, infection starts on the oldest or first-initiated leaves and progresses upward with maturity of the plant. Leaf loss ranges from 25 to 66% of the total leaves initiated.

Early in the investigation of the Stemphylium disease of trefoil, approximately 400 pounds of forage was determined lost when 66% of the total initiated leaves were defoliated.

After over a hundred thousand trefoil seedlings were screened for resistance to Stemphylium leaf spot, the degree of resistance was found to be low and complex. Hence, a temporary method of suppressing the diseases was undertaken until a high degree of resistance could be found.

In preliminary tests with various fungicides and antibiotics, 2,4-dichloro-6-(o-chloroanilino)-s-triazine (Dyrene[3]) was selected as the most promising in suppressing the Stemphylium disease. During the 1959, 1960, and 1961 growing seasons, further studies were made with Dyrene to test its effectiveness in suppressing Stemphylium infection when the fungicide was applied at three different concentrations and time intervals. A preliminary report has been given[4].

MATERIALS AND METHODS

Small, 6- x 7-foot plots of 1-year-old trefoil plants were used for the study.

Beginning April 24, 1959, three plots were treated at 7-day intervals, another three at 14-day intervals, and still another three at 21-day intervals throughout the growing season with 3 pounds of active Dyrene in 200 gallons of water/acre. One untreated check plot was used for each application interval. By use of the same method and design, another group of plots was

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[1] Cooperative investigation of Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Virginia Agricultural Experiment Station. Published with the approval of the Director of the Virginia Agricultural Experiment Station.

[2] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Blacksburg, Virginia.

[3] Dyrene, a fungicide, was manufactured and contributed by Chemagro Corporation. Mention of a specific name is for identification and does not imply any endorsement by the United States Department of Agriculture or Virginia Agricultural Experiment Station.

[4] Drake, C. R. 1962. Effectiveness of Dyrene for control of Stemphylium leaf spot of birdsfoot trefoil. (Abst.) Phytopathology 52: 8.

Table 1. Effectiveness of different concentrations of Dyrene, when applied at different time intervals, in suppressing Stemphylium leaf spot infection of birdsfoot trefoil, and its influence on yield for 3 consecutive years.

Year and concentration of Dyrene/acre (in pounds)	Time intervals of application[a] (days)	Annual av. no. leaves/plant/harvest: initiated	infected by S. loti	defoliated	Total annual dry weight yield/acre (in pounds)
1959					
3	7	13.8	4.6	2.6	6891
do.	14	14.2	4.8	2.6	7193
do.	21	14.1	5.0	2.7	6200
Untreated		14.0	5.7	3.5	6128
4.5	7	14.1	4.4	2.7	6141
do.	14	14.3	5.0	2.6	7495
do.	21	14.5	5.0	2.6	7705
Untreated		14.0	5.7	3.5	6804
6	7	14.1	4.0	2.1	7608
do.	14	14.7	4.4	2.3	8243
do.	21	14.3	4.4	2.6	8751
Untreated		14.0	5.7	3.5	6420
1960					
3	7	13.7	2.1	1.6	7103
do.	14	14.1	2.5	1.6	6544
do.	21	14.7	3.1	2.5	5904
Untreated		14.5	6.5	4.8	5491
4.5	7	14.4	2.0	1.5	6722
do.	14	13.7	2.2	1.6	6418
do.	21	14.4	3.1	2.2	5891
Untreated		14.6	6.9	5.2	5406
6	7	14.4	1.5	1.5	6664
do.	14	14.7	2.5	2.1	6508
do.	21	14.0	2.3	1.8	6878
Untreated		14.4	7.2	5.3	5485
1961					
3	7	13.9	1.8	1.4	11,748
do.	14	14.1	2.9	1.9	10,680
do.	21	14.0	4.2	2.8	8,756
Untreated		13.5	6.6	5.7	7,112
4.5	7	13.6	2.0	1.1	12,016
do.	14	14.0	3.5	2.8	10,546
do.	21	13.7	3.7	2.4	8,320
Untreated		13.9	7.1	6.1	7,452
6	7	13.3	1.1	0.8	12,298
do.	14	13.8	3.0	2.0	10,656
do.	21	13.1	3.6	2.7	9,376
Untreated		13.4	6.9	5.9	7,372

[a] Three replicates for each application time interval within a concentration.

treated with 4.5 pounds of active Dyrene/acre, and still another group with 6 pounds of active Dyrene/acre. The fungicide was applied with a knapsack sprayer, equipped with off-center double swivel TeeJet nozzles and 02 flat spray tips.

The leaves initiated and the leaves infected and defoliated by S. loti were counted on 25 plants at random in each of the treated and untreated plots just before harvest to determine the effectiveness of the fungicide.

Yields were determined by harvesting a 3-foot strip across each treated and untreated plot. The green forage was dried to a constant weight in a forage-drying chamber maintained at 180° F.

Table 2. Average of the effectiveness of different concentrations of Dyrene, when applied at different time intervals for 3 successive years, in suppressing Stemphylium leaf spot infection of birdsfoot trefoil, and its influence on yield (average 1959, 1960, and 1961 data).

Concentration of Dyrene/acre (in pounds)	Time intervals of application[a] (days)	Annual av. no. leaves/plant/harvest			Total annual dry weight yield/acre (in pounds)
		initiated	infected by S. loti	defoliated	
3	7	13.8	2.8	1.8	8580
do.	14	14.1	3.1	2.0	8139
do.	21	14.3	4.1	2.6	6953
Untreated		14.0	6.3	4.7	6243
4.5	7	14.0	2.8	1.7	8293
do.	14	14.0	3.5	2.3	8153
do.	21	14.2	3.9	2.4	7305
Untreated		14.1	6.6	4.9	6487
6	7	13.9	2.2	1.4	8856
do.	14	14.4	3.3	2.1	8469
do.	21	13.8	3.4	2.3	8335
Untreated		13.9	6.6	4.9	6425

[a] Three replicates for each application time interval within a concentration.

RESULTS

The results were not very striking during 1959 but all treatments of Dyrene reduced the incidence of the disease from that in the untreated plots (Table 1). The initial infection had already occurred in 1959 before the first application of Dyrene was made, hence the effectiveness of Dyrene in suppressing the disease was greatly reduced. Unfavorable environmental conditions for infection in mid- and late summer also reduced the overall quantitative efficiency of the fungicide.

In 1960 and 1961, the first application of Dyrene was made March 15 before the initial infection. Conditions were good for early and late infection also, therefore results were more pronounced for both of the last 2 years.

Only three harvests were made during the 1960 growing season because of a prolonged drought in mid-summer. The average number of leaves infected and defoliated, respectively, was 2.9 and 2.8 times greater for the untreated plots than for an average of all the treatments (Table 1). Significant increases in yields were obtained with all concentrations of Dyrene at all application intervals (Table 1).

Owing to a long favorable growing season, four harvest were made during 1961. In every instance during 1961, as the application interval increased the effectiveness of the fungicide decreased (Table 1). Similarly, as the number of infections increased the total annual yield decreased (Table 1).

In 1961, one treated plot (6 lb/acre at 14-day intervals) and one untreated plot were not harvested until August 16 for comparison of the effectiveness of the fungicide. At this time, the over-mature plants in the treated plot were still covered with healthy green leaves. In contrast, however, the disease-ridden, over-mature plants in the check plot were almost defoliated and the few remaining leaves were brown and non-functional.

Application of 6 pounds/acre of Dyrene at weekly intervals increased total average annual forage yield during 3 consecutive years by 2431 pounds/acre over untreated plots (Table 2). The same treatment, however, during 1961 increased total average annual yield by 4926 pounds/acre over untreated plots (Table 1). The increase in yield was due to ideal conditions for plant growth, fungus infection, fungicide activity, and a reduction of the stand in the untreated plots. At the end of the 3-year test there was an average of 55% stand in the untreated plots in comparison with 88% stand in the Dyrene-treated plots.

DISCUSSION

There was considerably more cover left on the Dyrene-treated plots after harvest than on the control plots. Green leaves remaining on the stubble of the plants in the Dyrene-treated plots provided protection to the crown of the plants from direct sunlight and the sudden increase in temperature after harvest. In contrast, no leaves remained on the stubble of the plants in the untreated plots to provide cover for the sensitive crowns after harvest.

In the Southeast, trefoil stands are severely reduced in number of surviving plants after 3 to 4 years. Heretofore, the Stemphylium disease has been implicated as causing only a loss in yield and quality of forage; but through defoliation of the older leaves, it also may be a factor in contributing to the loss of plants by permitting crown exposure to direct sunlight and the resulting high temperature after harvest.

At present, fungicides are probably not economical for use on forage crops. They are, however, an excellent tool for determining actual forage loss caused by foliage diseases.

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SPRAY PROGRAMS AND THE CONTROL OF SOOTY BLOTCH OF APPLES[1]

Robert H. Daines[2]

Summary

In sooty blotch (Gloeodes pomigena) control experiments, folpet and glyodin were more effective than dodine, zineb, captan, or a mixture of captan and zineb. Fungicides used with lead arsenate proved to be considerably more effective than combinations of these same fungicides and dichlorodiphenyltrichloroethane (DDT), O,O-dimethyl S-(4-oxo-1,2,3-benzotriazinyl-3-(4H)-methyl) phosphorodithioate (Guthion), or 1-naphthyl N-methylcarbamate (Sevin). Apparently lead arsenate exerts considerable fungicidal activity against G. pomigena.

INTRODUCTION

In New Jersey, where many growers discontinue a regular apple fungicide program by early July, sooty blotch caused by Gloeodes pomigena may become a problem that requires special spray applications. This problem is intensified when lead arsenate is dropped from the spray program and by the comparative ineffectiveness of captan against this disease. To avoid undue losses from sooty blotch, one or more applications of a mixture of captan and zineb are commonly applied during the fall months. This mixture has found favor in a summer and fall spray program because of its reported (3) value against Botryosphaeria rot despite its seemingly mediocre performance against sooty blotch. Since folpet is as effective as the captan-zineb combination against Botryosphaeria rot, it seemed important to determine its comparative value in a sooty blotch control program. During the period when sprays are of value in combating scab, sooty blotch infections occur, so folpet, dodine, captan, zineb, and glyodin were tested both in combination with insecticides during the first brood coddling moth period and alone as special sooty blotch sprays.

PROCEDURE

The sprays were applied on Red Delicious apples in 1959 and 1960 during the first brood coddling moth period. These applications were made on a 10-day schedule with the fifth cover spray occurring about the first of July. In both years, disease records were made during the first week in September. The rainfall during the summer and fall months for both years of this test was below normal.

During the fall of 1961, special sooty blotch sprays were applied to Golden Delicious apples in two plantings. These tests were designed to determine the value of special sooty blotch spray applications.

In all experiments reported, four or five randomized single apple trees of medium size were sprayed from the ground, using an 8-cluster nozzle boom and 500 pounds of pressure.

RESULTS

Tables 1 and 2, which consider the main effects of the fungicides and insecticides used, show folpet and glyodin to be the most effective fungicides, with dodine intermediate in effectiveness, and captan exhibiting the least activity against G. pomigena. Among the insecticides included in this test, lead arsenate demonstrated real value in a sooty blotch control program. These data also indicate that where lead arsenate was used with effective fungicides (folpet and glyodin) during the latter part of May and June, sooty blotch was not a problem on early-fall-harvested apples (Red Delicious) during the years these tests were in progress. Where, however, these same fungicides were used with organic insecticides, the incidence of sooty blotch was sufficiently severe to cause concern.

Since sprays applied during May and June may not always provide enough protection against sooty blotch occurring on the fall crop, several fungicides were applied during the fall period to Golden Delicious apples in sooty blotch control experiments. No insecticides were used in

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Table 1. The effect of fungicide-insecticide combinations used during May and June on the incidence of sooty blotch on Red Delicious apples at harvest*.

Treatment	Disease %	Level of significance 1%**
Folpet 2# second cover, 1# thereafter, lead arsenate 3#	2.8	a
Glyodin 1 1/2 qt second cover, 3/4 qt thereafter, lead arsenate	4.1	a, b
Dodine (Cyprex) 1/2#, lead arsenate	6.8	a, b, c
Captan 2# second cover, 1# thereafter, lead arsenate	11.0	a, b, c, d
Folpet, Guthion 1 1/4#	13.2	b, c, d
Folpet, DDT 2#	14.4	b, c, d
Glyodin, Guthion	14.9	c, d
Glyodin, DDT	16.1	c, d, e
Folpet, Sevin 1#	18.1	c, d, e
Glyodin, Sevin	21.4	d, e
Dodine (Cyprex), Guthion	21.8	d, e
Dodine (Cyprex), DDT	22.5	d, e
Dodine (Cyprex), Sevin	31.6	e, f
Captan, DDT	44.8	e, g
Captan, Sevin	54.4	g
Captan, Guthion	58.1	g

*Percentages converted to angles for calculations. Angle = $\arcsin \sqrt{\%}$

**Treatments followed by same letter do not differ at 1% level of significance according to Duncan's multiple range test.

Table 2. Main effects of fungicides and insecticides in the sooty blotch control experiment reported in Table 1*.

Treatment	Disease %	Level of significance 1%**
Folpet 2# second cover, 1# thereafter	11.9	a
Glyodin 1 1/2 qt second cover, 3/4 qt thereafter	14.1	a, b
Dodine (Cyprex) 1/2#	20.7	b
Captan 2# second cover, 1# thereafter	42.1	c
Lead arsenate 3#	6.2	d
DDT 2#	24.5	e
Guthion 1 1/4#	27.0	e
Sevin 1#	31.4	e

*Percentages converted to angles for calculations. Angle = $\arcsin \sqrt{\%}$

**Treatments followed by the same letter do not differ at the 1% level of significance according to Duncan's multiple range test.

these applications, and the orchards used had not received an arsenical application since the second cover. At the time of application sooty blotch was not visible on the apples.

The first experiment involving a special sooty blotch application consisted of one preharvest spray applied 5 weeks before harvest and data-taking (Table 3). In this experiment folpet and glyodin again proved to be most effective against this disease, captan-zineb were intermediate in effectiveness, and captan showed little value on a 5-week program.

In a second special sooty blotch program, applications were made 2 months and 1 month before harvest (Table 4). Although no sooty blotch was visible at the time of the second spray application, it did begin to show shortly thereafter.

Table 3. Comparative value of fungicides applied 5 weeks before harvest for sooty blotch control[a, ***].

Materials used per 100 gallons	% fruit showing sooty blotch	Level of significance 1%**
1. Folpet 1 1/2#	7.0	a
2. Glyodin 3/4 qt	9.1	a
3. Captan 1#, zineb 1#	17.0	b
4. Zineb 1 1/2#	24.3	b
5. Captan 1 1/2#	43.1	c
6. Check, unsprayed	71.4	d

[a] Golden delicious apples used in this test harvested October 12, 5 weeks after the fungicides listed in this table were applied.

**Treatments followed by the same letter do not differ at the 1% level of significance according to Duncan's multiple range test.

***Percentages converted to angles for calculations. Angle = arcsin $\sqrt{\%}$

Table 4. Comparison of fungicides and time of application in sooty blotch control[a, ***].

Materials used per 100 gallons	Application dates: Aug. 8	Application dates: Sept. 5	% fruit showing sooty blotch	Level of significance 1% **
1. Folpet 1 1/2#	+	+	0.2	a
2. Glyodin 3/4 qt	+	+	1.0	a
3. Captan 1#, zineb 1#	+	+	4.1	a
4. Zineb 1 1/2#	+	+	10.5	b
5. Folpet 1 1/2#	+	-	13.5	b
6. Glyodin 3/4 qt	+	-	17.6	c
7. Captan 1#, zineb 1#	+	-	28.8	d
8. Zineb 1 1/2#	+	-	39.5	e
9. Check, unsprayed	-	-	66.7	f

[a] Golden Delicious apples used in this test were harvested on October 5.

**Treatments followed by the same letter do not differ at the 1% level of significance according to Duncan's multiple range test.

***Percentages converted to angles for calculations. Angle = arcsin $\sqrt{\%}$

CONCLUSIONS

From the experiments reported above, folpet (a fungicide discovered and orchard tested along with captan by Daines and Kittleson (1, 2) in a joint Standard Oil-Rutgers project) and glyodin demonstrated real value in sooty blotch control. Where lead arsenate was used with a fungicide, the control of sooty blotch was greatly improved. Although glyodin shows real value in a sooty blotch program, weakness against other summer diseases has limited its use.

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EXPERIMENTS WITH DBCP IN ESTABLISHED VINEYARDS

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Summary

DBCP has been tested in 14 established vineyards at rates ranging from 1.25-10 gallons/acre of active ingredient. Tests included application by drills on machine-driven rigs, flooded in irrigation water and by sprinklers. Marked reductions in numbers of nematodes have been found at certain rates but the populations built up again within 3 to 5 months. In one test vegetative growth was decreased at the higher rates.

Pronounced stronger vegetative growth resulted from treatments in two varieties, Tokay and Emperor. However, in the latter test production of fruit buds was adversely affected, giving lowered yields despite strong vegetative growth. Delay in maturity of fruit was important in both Emperor vineyards tested. Statistically significant increases in yield were obtained in three tests.

Declines in growth and yield of grapevines due to plant parasitic nematodes are becoming increasingly prevalent and are the cause of major economic losses to grape growers throughout California. Removal of infected vines followed by fumigation and replanting with clean stock is often the only feasible control measure, especially in cases where plant injury is particularly severe. However, an effective side-dressing treatment would be preferred, chiefly because of the high cost of treating and replanting, but also because the rapid reinvasion of fumigated replant soils leaves considerable doubt as to the long-range effectiveness and success of such control measures (3, 4, 5, 6).

Most nematocides which are economical when used under field conditions are phytotoxic to grapes at dosages effective for the control of nematodes. However, 1,2-dibromo-3-chloropropane (DBCP) has been shown to be highly effective as a nematocide and can be used safely at nematocidal dosages on many plants. Some annual crops such as tobacco, onion, and garlic are quite sensitive to DBCP (2), but many perennial crops have been treated without injury. One limitation reported by Lear and Raski (1) is the failure of DBCP to penetrate non-rotted tomato and grape roots to kill root-knot nematodes.

Experiments with DBCP in vineyards were started in 1954. Some of the first exploratory tests were made on single vines at various dosages to determine phytotoxic levels. Repeated trials applied by hand injectors on Tokay, Grenache and Thompson seedless varieties at rates varying from 2 1/2 to 10 gallons/acre of active ingredient produced no visible effects favorable or unfavorable.

Chisel Applications: The first test by machine-driven equipment was made near Fowler on Grenache vines growing on their own roots. The soil type was a light sand with a moisture holding capacity of about 3%. The predominant nematode species present was Meloidogyne incognita. A few specimens of Xiphinema americanum and Trichodorus sp. were also found in some soil screenings.

Forty vines in each of two adjacent rows were treated with 2 1/2 and 5 gallons/acre of DBCP respectively on March 4, 1954. The rows were 12 feet wide and the application was made by five chisels at 1-foot spacing along both sides of each row. The inside chisel was maintained about 12 to 18 inches away from the vine row and the chemical placed about 8 to 10 inches deep. The soil was packed by a roller-packer after treatment.

On July 15 (7 weeks after treatment) roots and soil were collected by shovel from several locations and composited into one large sample in each treated row plus one untreated row. In the laboratory the roots were sifted from the soil, washed clean of soil, and divided into portions, four per treatment approximately equal in size. The roots of each portion were cut into small pieces and placed in sterile soil in a clay pot. The separated field soil was mixed and a 400-cc aliquot placed in each of five pots. Tomato seedlings were transplanted to each pot and gall counts on the roots made 42 days later. These data showed a marked reduction of root-knot nematodes in the soil but not in the root sections treated at 2 1/2 gallons/acre. Almost complete control in both roots and soil was obtained at the 5-gallon/acre rate. Soil samples taken 31 weeks after treatment showed root knot had reinvaded the soil of both levels of fumigation.

The following year three replicated test plots of a size sufficient to secure yield records were established and records were obtained in each trial over a 3-year period beginning the year of treatment (Table 1). A pronounced improvement in growth was found only in Test 1 and in that experiment the effect persisted through all 3 years observed. Yield responses paralleled the vegetative growth and were much greater at the 5- and 10-gallon rates.

Soil samples taken in plot 2 on three different occasions by soil tube to a depth of 4 inches were tested for root-knot nematode in a manner similar to that described earlier, and in addition counts of Pratylenchus vulnus were made from wet screenings of separate aliquots of soil. Results are shown in Table 2.

One of the difficulties of injecting chemicals into the soils of established vineyards is the inability to treat close enough to the vine row to ensure complete coverage of soil areas. There is the threat of breaking large arms or damaging the trunk itself, and root pruning by the chisels is also undesirable. Most applications are especially difficult if the cane growth is very strong and presents a possible source of injury to the operator where working close to the vines. The formulation of DBCP in emulsifiable concentrates made possible the treatment of soil in irrigation water as flood applications or in sprinkler systems, eliminating most of the above objections. Furthermore, it may be possible to incorporate irrigation treatments into normal cultural operations.

Flood Applications in Irrigation Water: In the spring of 1960 a series of experiments were established in widely separated areas to test the application of DBCP in water on different grape varieties. The formulation used was a 50% emulsion but all reports here are converted to rates of active ingredient. In most tests the material was metered into a head stand which provided adequate time and agitation for the material to mix in the water before it emerged at valves at individual rows. The flow was high enough in two cases to treat all replicates of a particular rate at the same time. For all the other tests the flow was sufficient for treating only one replicate at a time. An attempt was made to time the flow to deliver enough water to wet the soil to a depth of 4 to 6 feet and DBCP was applied continuously during the flow of water. Pertinent data of each test are shown in Table 3.

Soil samples to 4- and 8-foot depths to test nematode control were taken from two of the plots, the results of which are shown in Table 4.

Yield records were obtained in 1960 in all trials, but in 1961 only three of the tests could be measured for yields. These results are shown in Table 5.

The yield results show no effects in Tests 4 and 5. Test 6 showed a reduction in yield in both years, with a much greater decrease in 1961. Yields in Test 7 were reduced in 1960 but not significantly in 1961. Yields in Test 8 were not significantly different in 1960 but all three treatments produced a marked increase in 1961. Still another effect was found in Test 9 in which yields increased in 1960 but were markedly decreased in 1961, especially at the 5- and 10-gallon rates.

Two very different and pronounced effects on growth were also noted. In plots 6 and 7 a delay in fruit maturity was general and much of the picking went into cull fruit. This was experienced despite a noticeable improvement in vegetative growth of the vines in plot 6 in 1961. In 1961 the treated vines at all rates grew remarkably strong yet yield results were reduced inversely proportional to the rate of chemical used. Apparently the shoots were sterile, resulting from poor fruit bud differentiation in the summer of 1960. The same response in vegetative growth was not found in Test 7.

The other effect was found in Test 9 in which the yield was increased with no effects noticeable on the vines in 1960. The next year, however, a count of the number of flower clusters per vine was made on March 24, 1961. A marked reduction was found in a proportion similar to the decrease in yield. These vines were pruned to two-bud spurs and examination revealed that one or both of these two distal buds were damaged or killed. The failure of these to push delayed the growth until the less fruitful basal buds developed shoots. In some cases the distal buds grew but produced distorted or irregular shoots. The same decrease proportional to increasing rates was true of bunch counts per vine made May 19, 1961. The yield results (Table 5) were significantly less at both the 5- and 10-gallon/acre rates. The general growth was distinctly depressed in the vines treated with 10 gallons/acre, especially in the weaker areas of the vineyard.

Sprinkler Application: A vineyard in the Livermore valley infested with Xiphinema index and Pratylenchus neglectus was treated on October 26-30, 1960 with 2 1/2 and 5 gallons/acre of DBCP applied through a sprinkler system. Another treatment included a 2 1/2 gallons/acre rate applied in October and repeated in June 1961. The soil type is very gravelly or rocky with a fairly high moisture holding capacity difficult to measure because of the high percentage of granite rocks in the soil. The plot extended through two varieties, Semillon and Muscat Cannelli, both old plantings which had been good producers but had declined seriously in recent years.

Table 1. Average yields in vineyards treated in 1955 with DBCP drilled into the soil by machine.

Test number	Nematode species present	Treatment (gallons/acre)	Average pounds/vine 1955	1956	1957
1. Tokay var. (16-year-old vines in sandy loam soil; treated Mar. 14; soil temperature 51° F; soil moisture 8.2-9.1%)	*Meloidogyne incognita*	Check	27.6	12.1	10.9
	Pratylenchus vulnus	2 1/2	32.2*	17.4*	18.4*
	Xiphinema index	5	36.6**	20.3**	20.5**
	Criconemoides xenoplax	10	38.6**	21.9**	26.9**
	Paratylenchus hamatus				
2. Grenache var. (16-year-old vines in sandy soil; treated Jan. 26; soil temperature 46° F; soil moisture 4.7%)	*Meloidogyne incognita*	Check	41.8	39.1	29.1
	Xiphinema americanum	2 1/2	48.9	45.1	32.7
	Trichodorus sp.	5	43.8	45.0	31.9
3. Carignane var. (8-year-old vines in loamy fine sand; treated Apr. 4; soil temperature 58° F; soil moisture 9.1-9.4%)	*Meloidogyne incognita*	Check	44.0	29.2	36.8
	Pratylenchus sp.	2 1/2	44.3	29.9	37.5
		5	45.1	28.0	35.0
		10	43.6	21.6**	40.1

*Statistically significant at 5% level.
**Statistically significant at 1% level.

Table 2. Average nematode counts in the top 4 feet of soil in Test 2 (Grenache vines near Fowler) treated with DBCP drilled in the soil.

Treatment	April 15, 1955		July 6, 1955	March 1, 1957
(gallons/acre)	root-knot galls	root-lesion	root-knot galls	root-knot galls
Check	79.0	16.0	226	88
2 1/2	1.5**	1.4**	94	101
5	0.0**	0.0**	37	132

**Statistically significant at the 1% level.

Table 3. 1960 flood applications of DBCP in irrigation water.

	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9
Variety	Tokay	Red Malaga	Emperor	Emperor	Thompson seedless	Perlette
Age of vines	30 years (?)	13 years	8 years (?)	15 years	22 years	6 years (?)
Soil type	Hanford sandy loam	Dinuba fine sandy loam	Exeter loam	San Joaquin sandy loam	Hesperia loamy fine sand	Coachella fine sand
Date of treatment	April 20-21	March 3-4	April 6-10	May 9-11	March 9-10	February 25
Moisture equivalent	4.5 - 6.6%	9.1 - 10.3%	13.8 - 14.1%	19.5 - 31.0%	9.1 - 9.3%	--
Moisture content	5.0 - 6.6%	5.8 - 8.4%	9.0 - 9.3%	--	6.1 - 9.1%	--
Nematode species	*Meloidogyne incognita* *Xiphinema americanum*	*Meloidogyne arenaria thamesi* *Xiphinema americanum* *Criconemoides xenoplax*	*Pratylenchus vulnus*	*Xiphinema index* *Meloidogyne* sp.	*Meloidogyne incognita* *Xiphinema americanum* *Paratylenchus hamatus*	*Meloidogyne javanica*

Table 4. Average nematode counts from vines treated with DBCP flooded in irrigation water.

Treatment (gallons/acre)	Test 4 23 days after treatment	Test 5 35 days after treatment	 132 days after treatment
Check	34.7	197.2	207.0
1 1/2	55.7	5.8**	152.2
2 1/2	27.7	1.4**	135.4
5	51.4	1.0**	74.0
Sampling depth	0-8 ft.	0-4 ft.	0-8 ft.

**Statistically significant at the 1% level.

Table 5. Average yield in pounds/vine in 1960 flood irrigation tests with DBCP.

Treatment	Test 4	Test 5	Test 6		Test 7[b]		Test 8		Test 9	
(gallons/acre)	1960	1960	1960[a]	1961	1960	1961	1960	1961	1960	1961
Check	22.1	53.6	44.3(5.9)	27.8	11.4	11.0	39.6	36.9	22.6	36.7
1 1/2	24.9	46.5	--	--	--	--	36.3	47.2**	--	--
2 1/2	24.9	47.0	42.4(8.3)	25.2	--	--	38.3	47.1**	25.7**	32.8
5	26.0	46.4	40.0(10.3)	14.1	9.1	10.0	39.5	43.4**	25.0**	26.4**
10	--	--	36.0(15.4)	9.5	6.7	9.3	--	--	25.2**	17.2**
5 + 5 May & July	--	--	--	--	8.3	12.1	--	--	--	--

**Statistically significant at 1% level.

[a] Average of total yields are outside the parenthesis; part of this total (inside parenthesis) was cull fruit resulting from late maturation.

[b] The fruit in both years in Test 7 was late maturing resulting in much cull fruit. Separate records of these could not be obtained but over the entire plot the average per vine cull fruit in the plot was approximately 22 pounds in 1960 and 3 pounds in 1961.

Table 6. Average nematode counts from vines treated with DBCP by sprinkler application.

Treatment (gallons/acre)	Average counts/400 cc soil							
	February 1961				January 1962			
	X. index		*P. neglectus*		*X. index*		*P. neglectus*	
	0-2'	2-4'	0-2'	2-4'	0-2'	2-4'	0-2'	2-4'
Check	42.1	41.5	32.6	39.8	107.2	81.9	100.4	48.9
2 1/2 - Oct.	6.8	13.1	40.6	4.6	24.5	19.9	70.5	56.9
2 1/2 - Oct. & June	-	-	-	-	4.7	10.9	75.0	42.9
5 - Oct.	0.2	8.6	53.2	8.8	12.2	60.4	195.4	15.6

Table 7. Average yields from vines treated with DBCP by sprinkler application.

Treatment (gallons/acre)	Average pounds/vine			
	Semillon variety		Muscat Canelli variety	
	pretreatment 1960	posttreatment 1961	pretreatment 1960	posttreatment 1961
Check	3.8	8.3	3.5	6.4
2 1/2 - Oct.	3.9	11.2*	4.0	9.5*
2 1/2 - Oct. & June	3.9	10.0	4.6	9.3
5 - Oct.	2.8	8.3	3.5	7.2

*Statistically significant at 5% level.

Most of the chemical was applied in a short period of time at the first part of the sprinkling by injection into the main line. The sprinkling procedure followed was to presoak the soil for 1 hour, then 25 minutes for application of 90% of the material, 15 hours of water alone, 3 minutes during which 10% of the dosage was applied, and finally 26 minutes of water alone finished the treatment.

Soil samples to measure nematode control were collected twice by a mechanical soil auger. The rocky nature of that soil type made it impossible to sample by hand auger or soil tube and nearly so by pick and shovel. The mechanical auger partly mixed the soil as it was dug but a fairly good separation by depth was possible by taking the sample from the auger blade itself after it was raised. Two levels were sampled approximately 0-2 and 2-4 feet in depth with results of these samplings shown in Table 6.

Yield records were collected in 1960 before the treatments were applied and 1961, the first year after. These records are shown in Table 7. The most notable effect is a statistical increase in yield at the 2 1/2 gallon/acre rate in 1961.

DISCUSSION

The effectiveness of DBCP as a nematocide is confirmed in most of these tests, although for some reason control of root knot was poor in Test 4. Buildup of nematode populations usually occurred within 3 to 5 months after treatment even where control was quite good. This may be explained by the failure of DBCP to effect nematode control inside root tissues and these surviving nematodes serve as the source of reinfestation of soil and roots.

Vegetative growth of vines was affected in three of the nine tests, but in the others no discernible differences were found. Treated vines in two of the three tests showed stronger vegetative growth, but in the third, growth was depressed.

Regardless of the effects of DBCP on nematode populations or vegetative growth the final judgment of its practicality as a control can only be based on improvements in yield and/or quality of grapes. It is generally expected that differences in production will be accompanied by similar differences in vegetative growth. Yet in Test 8 yield increases were recorded in 1961 without any noticeable differences in vine growth. Also, the treated vines in Test 6 which showed remarkably strong vegetative growth actually produced less fruit. Increases in yield were obtained in three tests of the nine reported but judged in terms of cost only two approach success economically. DBCP in 25% or 50% formulations is sold for approximately $20/gallon of active ingredient and the dosages tested would cost from $25 to $200/acre. An increase in yield in pounds/vine needed to achieve an economical return is difficult to calculate and will vary in each vineyard depending upon the variety grown and whether it is produced for fresh pack, raisins or the winery. In small experimental plots it is difficult to obtain accurate records of the value of a crop because such tests are often picked entirely for the winery. There is no way of knowing how much of the fruit from such pickings would have been suitable for fresh pack, which brings a much higher return. Based on the lower return of winery prices, the lower rates of 1 1/4 or 2 1/2 gallons/acre in Tests 1 and 8 approach success economically. The carryover for three years in Test 1 is encouraging and yield records for 1962 will be most important in Test 8 to see if the improvement found in 1961 will hold again the second year. The rather common occurrence of ectoparasitic types of nematodes, especially the dagger nematodes, Xiphinema americanum and/or X. index, is interesting and suggests control of these species may account for the growth responses obtained. However, it is true that similar infestations were also present in Tests 2, 4 and 5 where no responses were found.

Acknowledgments: The invaluable assistance of many cooperators on these tests is sincerely appreciated because without their help the tests would not have been possible, including: A. N. Kasimatis, Extension Viticulturist, University of California, Davis; the following Farm Advisors: James Kissler, San Joaquin County; Curtis Lynn and Pete Christiansen, Fresno County; Frederik Jensen, Tulare County; Kenneth Hench, Kern County; Dean Halsey, Riverside County; and especially the growers who offered their vineyards for the tests.

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FOMES ANNOSUS ON SHORTLEAF PINE IN MISSOURI

F. H. Berry and O. J. Dooling[1]

Until last year, only one instance of shortleaf pine (*Pinus echinata*) mortality from *Fomes annosus* (Fr.) Cke. was known in Missouri[2]. In the spring of 1961, however, discovery of annosus-killed trees in a shortleaf pine plantation in Dent County prompted a survey to find out the extent and severity of this root rot. The survey was confined to State and Federal land in southern Missouri, which is within the natural range of shortleaf pine. The investigation revealed that *F. annosus* is widely distributed in this part of the State (Table 1).

Infection centers were found in 7 of the 13 national forest ranger districts examined and in both the State forests visited. Most infections appeared to be of recent origin. All infected stands had been thinned in the past, which corroborates previous research suggesting that thinning creates a favorable environment for the establishment of the disease organism.

F. annosus sporophores were found on either stumps or dead and dying trees in most of the thinned pine plantations examined and in about half of the thinned natural pine areas. Infection centers varied in size from a few to a dozen or more dead and dying trees. In natural pine stands damage from the fungus was less apparent than in plantations.

Damage from annosus root rot was most serious in the southern part of Palmer Plantation where approximately 75% of the trees were either infected or killed by the disease (Fig. 1). Sporophores were found on trees whose crowns appeared perfectly healthy, as well as on trees with thin crowns and yellow needles. Pine reproduction in the seedling stage was also affected. The Palmer Plantation is on a moderately well-drained low slope with a westerly aspect. The soil is a silt loam with an average pH of 5.6.

Table 1. Distribution of *Fomes annosus* in shortleaf pine tracts examined in Missouri.

Name of tract	County	Size of tract (acres)	Origin of trees	Age of trees (years)	Year thinned
Palmer Plantation	Washington	23	planted[a]	26	1952, 1957
Turtle Plantation	Dent	36	planted	26	1953 - 1954
Blue Hole Plantation	Ripley	15	planted	26	1956, 1958
Lawson Hollow Plantation	Shannon	25	planted	22	1951
Seed Production	Oregon	70	natural	47	1941, 1953
Pomona Plantation	Howell	135	planted	25	1939
Seed Production	Douglas	60	natural	41	1955
Merrill School	Texas	135	natural	40	1958
East End	Iron	100	natural	52	1956
Parker	Reynolds	10	natural	50	1951
Range Rider Plantation	Reynolds	7	planted	25	1954
Range Rider Stand	Reynolds	2	natural	40	1956
Reidsville Plantation	Franklin	26	planted	25	1956
Ruble Plantation	Franklin	0.2	planted	21	1951

[a] All plantings were 6 x 6 feet except Range Rider Plantation which was 8 x 8 feet.

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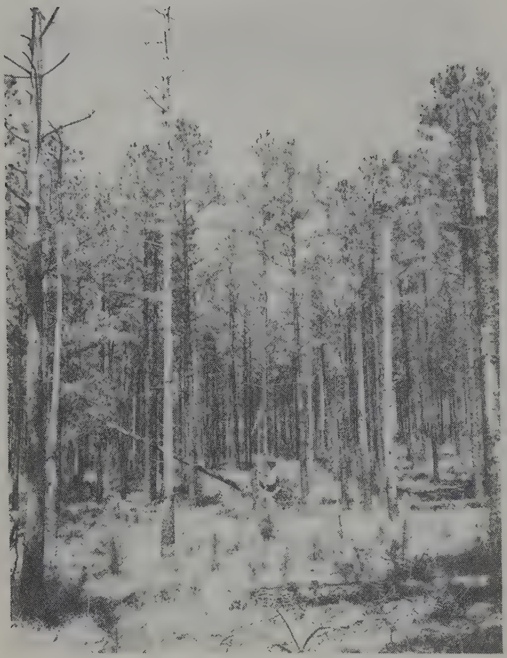

FIGURE 1. Mortality from F. annosus in a 26-year-old shortleaf pine plantation in Missouri. The plantation was thinned in 1952 and 1957.

Earlier research has shown that practically all losses from annosus root rot take place after thinning. Conditions in the absence of thinning do not seem to favor the disease organism since no losses in Missouri are known from unthinned stands. Annosus root rot will become a more serious management problem when the many shortleaf pine plantations in this region reach thinning age.

CENTRAL STATES FOREST EXPERIMENT STATION, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, COLUMBIA, MISSOURI

INTERRELATIONSHIPS OF BUD-PERPETUATED CONDITIONS IN PRUNE TREES, AND VARIATIONS IN THESE CONDITIONS WITH TIME[1]

A. W. Helton[2]

Summary

Buds from a sample of 13 prune source trees were budded to virus-free Italian prune acceptor trees early in 1957; various samples were budded to Myrobalan, Muir, Lovell and Mazzard seedlings in a greenhouse and in a nursery during 1957-60; annual and seasonal variations in symptom expression were studied in budded acceptor trees and seedlings, and in commercial orchard trees, during the 4-year period of the study.

The following conclusions were reached: 1) The various symptoms of the prune "leaf-spot complex" yield to each other in the same twigs in a poorly defined fashion. 2) Although most of the symptoms are associated, the underlying cause of these relationships has not been elucidated. 3) The so-called bud-perpetuation effect may or may not occur, with respect to continuation of source tree symptoms in donor-bud growth on other trees, but whatever symptom (or symptoms) develops in that growth is not likely to be constant year after year, nor even during a single year, and consequently is not truly bud-perpetuated. 4) Therefore, the concept of genetic deviation (that is, bud-perpetuation), as presently applied to prune abnormalities, is inadequate.

INTRODUCTION

Prune tree disorders have been under investigation in Idaho since 1951. During this period a number of influential factors have been studied, (3, 5, 7), but the one most intimately involved with the poorly-understood prune abnormalities has been found to be the virus-ringspot complex (4, 6).

Although ringspot viruses were common in young and old prune orchards and usually were present in trees showing the common abnormality symptoms, transmission studies conducted with the viruses established that they were neither directly nor immediately responsible for the symptoms generally exhibited by unthrifty orchard trees (6).

The abnormal symptoms of affected trees frequently have been reproduced on budded trees, in Idaho and elsewhere in the western United States, but only in subsequent growth from donor buds (1, 2, 5, 7). The ringspot viruses have been transferred to acceptor trees in these bud-perpetuation, or transmission, studies; but they have not been known to induce the original orchard-tree symptoms in inoculated trees within 1 to 5 years.

Annual surveys in Idaho since 1951 have revealed that a number of distinguishable conditions are of common occurrence in commercial prune orchards, but they have also raised questions concerning the constancy of these conditions in affected trees.

Although preliminary inoculation studies indicated that most such conditions were of a bud-perpetuated, or genetic, nature, variation was evident here also.

The study reported here was undertaken to clarify the interrelationships of these conditions and the effect of annual and seasonal changes in environment on symptom expression.

MATERIALS AND METHODS

Disorder Symbolism: To facilitate description of observed conditions, the following symbols are used: ALM, angular leaf mottle, mostly marginal; BLS, bulls-eye leaf spot, a generally circular chlorotic spot with necrotic center; FG, fruit gummosis, external and randomly arranged exudation of globules and/or tendrils of gum; FM, fruit malformation, in which the stem end is unusually tapered or the halves are unequal; GLC, green leaf curl, with margins rolling upward over the mid-rib, in the absence of mites; ILB, intervenous leaf burn, where recurrent necroses are prominent and irregular and without sharply delimited margins that are single or zonate in appearance; MLB, marginal leaf burn; TLB, tip leaf burn; MLS, mottle leaf spot, consisting of randomly arranged circular spots of chlorosis with diffuse margins,

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[1] Approved by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 548; study conducted as a part of Western Region Project W-64.

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with or without a rusty cast; MarLS, marginal leaf spot; ILS, intervenous leaf spot; RBLS, random-brown leaf spot, ranging from light and small spots 1 to 2 mm in diameter to dark and large spots up to 1 cm in diameter, with sharply delimited margins which may or may not have a zonate appearance; RIFN, random-internal fruit necrosis, in which necrotic pockets of tissue averaging 3 mm in diameter are scattered randomly in the fruit flesh; YLC, yellow leaf curl, with loss of green color, in the absence of mites; YLS, yellows leaf spot, in which the leaves turn yellow except for randomly arranged islands of greenish color; YLLS, yellow-leaf leaf spot, where randomly arranged brown spots are prominent in otherwise yellow leaves which drop off easily.

These conditions are not associated with nutritional disorders, per se, and are referred to collectively as the prune leaf-spot complex.

Bud-perpetuation on Prune Trees: In 1956, 13 trees showing various symptoms were selected from among those examined during the 1951-56 orchard surveys, for bud-perpetuation studies with prune acceptor trees (Fig. 1). Dormant budwood was collected from each donor tree in March 1957 and chip-budded to 2-year-old Italian prune trees (Prunus domestica) that had indexed virus-free on Shirofugen flowering cherry (P. serrulata). The budded trees were on Myrobalan plum (P. cerasifera) roots.

Bud-perpetuation on Seedlings: Studies of Prunus ringspot viruses in prune trees having raised the question of rootstock effects (4, 6), orchard samples were collected for bud-perpetuation studies in which various seedlings were used as acceptor trees.

In the spring of 1957, budwood was collected from six orchard trees, four of which had shown various symptoms the previous season (Fig. 2). These six source trees were tested on Myrobalan plum in a greenhouse and Lovell peach (P. persica) in a greenhouse and in a nursery.

In the spring of 1958, budwood was collected from six other orchard trees. Four of these source trees had shown various of the common symptoms the previous year, one tree had shown crinkle leaf symptoms, and one had exhibited no obvious symptoms (Fig. 2). Buds of these sources were grafted to Muir peach (P. persica) in a greenhouse.

In the spring of 1959, budwood was collected from five additional orchard trees, only two of which had exhibited various of the symptoms in question the preceding year (Fig. 2). These source trees were tested on Lovell peach in a greenhouse and Myrobalan plum in a nursery.

In the spring of 1960, budwood was collected from 23 orchard trees, all but six of which had shown one or more of the symptoms the previous year (Fig. 2). Seven of the source trees were tested on Lovell peach in a greenhouse, eight were tested on Mazzard cherry (P. avium) in a greenhouse, and eight were tested on Lovell peach in a nursery.

Time versus Symptom Expression in Source Orchards: Symptoms of 20 randomly selected prune trees in commercial orchards were recorded twice during 1959 (Fig. 3). In 1960, symptoms of 12 of the original 20 (8 had been removed), and of 20 additional trees, were recorded three times during the growing season.

Each tree was examined on all sides during each inspection, with symptoms being recorded if they were present in any part of the tree.

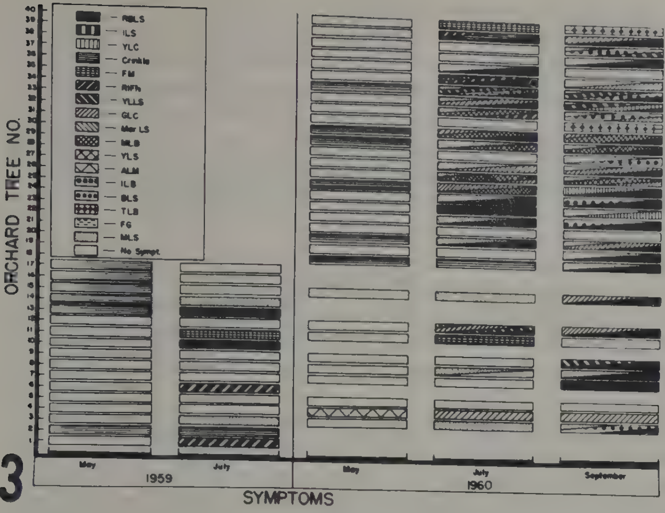
Time versus Perpetuation on Prune Trees: The 2-year-old Italian prune trees budded with 13 orchard sources in the spring of 1957 were continued under observation for 4 years.

Time versus Perpetuation on Seedlings: Sixteen orchard trees, 12 of which had exhibited symptoms in 1959, were sampled early in 1960; with budwood collected from 7 of them grafted to Mazzard seedlings in a greenhouse and Lovell seedlings in a nursery, and the remaining 9 grafted only to Lovells in a nursery (Fig. 4). Symptoms on donor bud growth were recorded three times during 1960.

RESULTS

Apparently normal donor-bud growth was produced with buds from all source trees investigated and from virus-free budwood used as controls. These results are not shown in Figures 1 to 4.

Bud-perpetuation on Prune Trees: Figure 1 shows that budwood taken from prune trees affected by the RBLS condition generally produced growth which also showed RBLS symptoms, as reported by other workers (2), but it also shows that the RBLS symptoms were not permanent in the donor-bud growth. (In the absence of a transmissible causal factor, co-identity with disorders reported by other workers can only be presumed on symptomatological bases.) Three of the four RBLS source trees represented in Figure 1 produced RBLS-affected bud growth, but in no case was the donor-bud growth continuously affected during the 4-year period of the study. (This has occurred repeatedly in Idaho during the past 10 years.)



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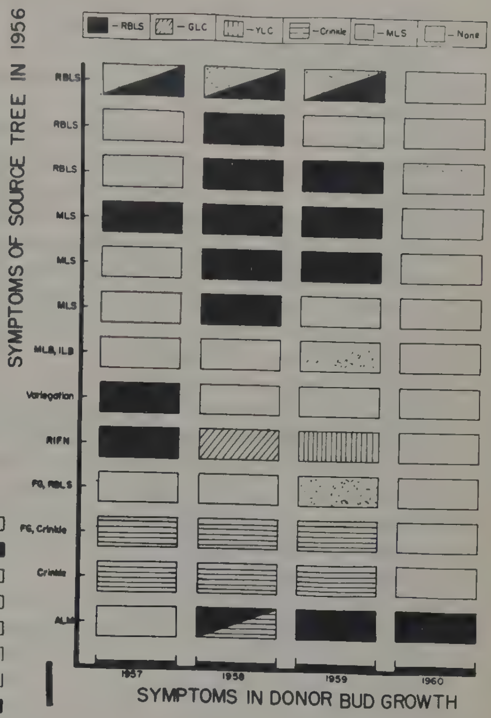

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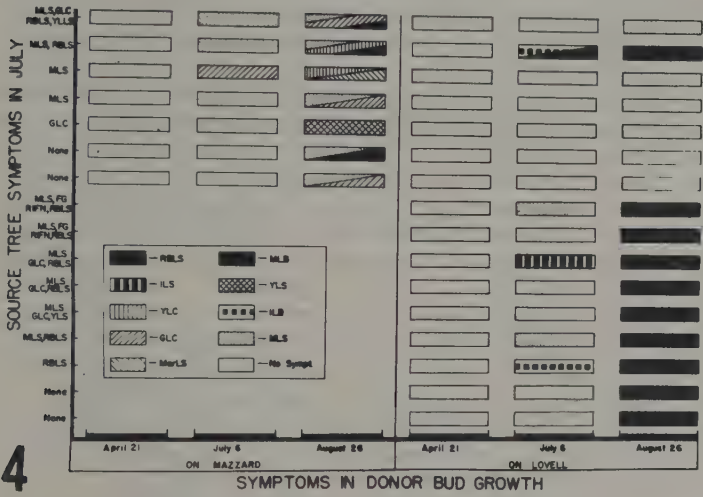

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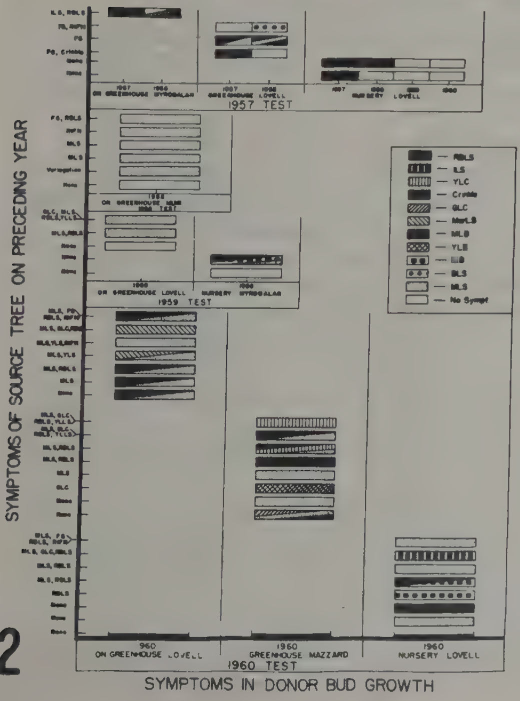

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FIGURE 1. Relationship of prune source tree symptoms to symptoms in growth from buds of those trees when grafted to virus-free Italian prune trees, and the effect of time on the bud-perpetuation response. (Three buds of each source chip-budded to each of two 2-year-old acceptor trees on Myrobalan roots in the spring of 1957; all symptoms recorded in July.)

FIGURE 2. Relationship of prune source tree symptoms to symptoms in growth from buds of those trees when the buds were grafted to peach, plum and cherry seedlings. (All symptoms recorded in July; three buds chip-budded to each of three 2-year-old seedlings in a greenhouse in March and a nursery in May 1957; three buds budded to each of four seedlings in March 1958; three buds budded to each of four seedlings in a greenhouse in March and a nursery in May 1959; three buds budded to each of three seedlings in a greenhouse in March and a nursery in May 1960.)

FIGURE 3. Seasonal variation in symptom expression of commercial Italian prune trees affected by a number of conditions not reproducible in budded acceptor trees.

FIGURE 4. Effect of elapsed time on symptom expression in donor-bud growth on 2-year-old Mazzard cherry seedlings in a greenhouse and Lovell peach seedlings in a nursery during 1960; three buds of each source chip-budded to each of three seedlings on March 29 (greenhouse) and May 8 (nursery).

Figure 1 also indicates a relationship among the symptoms represented by the source trees tested. This relationship is especially apparent for RBLS and MLS.

Bud-perpetuation on Seedlings: Several symptoms of the so-called bud-perpetuated type appeared in donor-bud growth on Lovell seedlings, both in the greenhouse and in the nursery, during the 1957 test (Fig. 2). As was the case in the perpetuation study on prune trees, however, these were neither permanent nor the same as the source tree symptoms.

Figure 2 also shows that in 1958 only the MLS symptom appeared on donor-bud growth on Muir seedlings, but this reaction occurred for every source that had shown any kind of symptom in the original tree the previous year.

In 1959, MLS again predominated in donor-bud growth.

In 1960, while MLS was the most common symptom on donor-bud growth on both Lovell and Myrobalan, other conditions (notably RBLS) were bud-perpetuated during that year. The interrelationships of the various symptoms again was indicated, by the fact that a number of the symptoms that appeared in donor-bud growth had not been evident in the individual source trees during the preceding year.

Time versus Perpetuation on Source and Budded Prune Trees: Since so much variation in symptom expression, from one type to another, was observed during the 1957 and 1958 studies, several orchard trees were selected for seasonal observation during 1959 and 1960.

Figure 3 verifies the reports of other workers (1, 2) in that spring growth was found to be relatively normal (crinkle leaf excepted). Very few of the symptoms of the complex were observed until July. During July, various of the symptoms were prominent and plentiful and still more so in September.

As was the case with the perpetuation studies described above, a close association was observed among the various distinguishable symptoms; an association which was especially apparent for MLS and RBLS.

The failure of the symptoms to be permanent and stable in affected growth is illustrated in Figures 1 and 3.

Time versus Perpetuation on Seedlings: Variation in symptom expression from one form to another also was observed when conditions of donor-bud growth on Mazzard and Lovell seedlings were recorded at three different times during 1960 (Fig. 4).

Even though the Mazzard trees were in a greenhouse where the environment was controlled, the "seasonal" effect still appeared in the form of increased incidence and severity, as well as change from one form to another, as the months passed. No symptoms were present in April, moderately severe forms appeared in July, and the necrotic symptoms (especially leaf-burn types) regarded as among the most extreme were prominent during late August.

DISCUSSION AND CONCLUSIONS

The so-called bud-perpetuation effect in prune trees needs a better definition. The term implies continuous symptoms in growth from affected buds, or in progeny trees that are produced from affected buds. The evidence submitted here indicates that such a continuity of perpetuation generally does not occur.

The interrelationships of most of the "leaf-spot complex" symptoms of the source trees were striking throughout all tests, both in the source trees and in growth from source-tree buds that were grafted to virus-free trees and to various seedlings. Various of the symptoms occurred together and/or alternated with each other in a more or less random fashion, with only the exception that the more severe forms tended to be more common toward the end of the growing season. The MLS and RBLS symptoms were prominently associated in this way, yet they often were found independent of each other; in different trees, and even in the same tree, they simply occupied different locations. (Fruit symptoms could not be evaluated in the few fruits produced on donor-bud growth.) Whether the fruit symptoms found on source trees is a phenomenon associated with Lovell roots (under the orchard trees) and not with Myrobalan roots (under the test trees) is not known.

These facts suggest that the various symptoms may have the same basic cause, with the variations being induced by associated factors as yet unidentified.

The annual variations in incidence of symptoms were apparent both in source trees and in donor-bud growth during the 4-year period of this study and are reflected in accompanying illustrations. This is symptom instability of a kind not compatible with the absolute term bud-perpetuation. Some seasonal variations might possibly be attributable to environmental fluctuations, but annual variations are not so easily dismissed.

Such seasonal variation in severity, and change from one form to another, also was evident. This transiency was apparent in nursery, plot, and commercial orchard trees, but it appeared also on budded seedlings under standardized greenhouse conditions (Fig. 2, 1957; Fig. 4, Mazzard). Therefore, this variation may prove to be more a function of physiologic processes of maturation (when influenced by the unidentified factors) than of environment change *per se*.

Mazzard is not a satisfactory rootstock for prune trees. This was reflected in the fact that symptoms which developed in donor-bud growth on Mazzard seedlings were more complex and prominent than the symptoms which appeared in growth from buds of the same source trees on Lovell seedlings (Fig. 4). This is regarded as additional evidence (6) that the prune leaf-spot complex arises more directly through root-scion stress effects than from any single direct-cause factor, whether the stress arises from poor compatibility *per se* (for example, Mazzard) or from reduced vigor of rootstocks due to factors (such as virus infection (6)) which may affect the rootstock more severely than the scion.

Thus, the evidence reported here supports these conclusions for the sources tested: 1) Prune leaf-spot complex symptoms which are not reproducible on budded prune trees within a reasonable period (for example, 4 years) with standard bud-grafting procedures generally are not truly bud-perpetuated. 2) Symptoms produced in growth from buds of an affected tree may be one or more of the symptoms of the complex and may or may not include the symptoms exhibited by the source tree. 3) Symptoms of the complex may at first appear to be bud-perpetuated but are not continuously evident in donor-bud growth, either in succeeding seasons or on succeeding years. 4) Individual symptoms of the complex are closely related to others, occurring with or alternating with them, and this relationship is particularly striking in bud-grafting studies involving MLS and RBLS symptoms. 5) Genetic and tissue-aging factors appear to be involved in the manifestation of various symptoms of the complex, but root-compatibility and root-thrift factors seem to be involved also and require further investigation. 6) The concept of bud-perpetuation of non-transmissible prune abnormalities is in need of reappraisal in view of the instability indicated for these abnormalities and particularly in light of the subtle effects which compatibility, infection, and other factors probably play in the incidence, fluctuation, and worsening of these conditions.

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STABILITY OF CRINKLE SYMPTOMS IN PRUNE TREES[1]

A. W. Helton[2]

Summary

An orchard of 676 Stanley prune trees and an orchard of 336 Italian prune trees were mapped and inspected on a tree-by-tree basis for 4 years. Seasonal variations in symptom expression in a single year were observed in one tree in each of seven other Italian prune orchards in 1959; and in one tree in each of seven additional orchards in 1960. Bud-perpetuation studies were conducted with seven different Italian trees over the same 4-year period.

Results revealed that 1) annual incidence of crinkle symptoms varies; 2) recurrence varies annually also; 3) symptoms may be present in a tree in the spring and not present later, only the severe forms being stable; and 4) normal-appearing growth can be produced by buds from crinkle-affected twigs, and vice versa.

INTRODUCTION

Since surveys of prune orchards were begun in Idaho in 1951, a condition similar to the crinkle leaf of sweet cherry trees (3) has been encountered in varying distribution patterns and varying degrees of severity. Descriptions of the two conditions (1, 2, 3) show that they are similar in appearance, though comparative information regarding their basic causes is lacking.

Variations in incidence of cherry crinkle leaf in affected orchards, and even in individual trees (2) raised questions concerning the behavior of the crinkle condition (1) in orchards of prune trees. During preliminary surveys carried out in 1951-1953, crinkle symptoms frequently were observed in certain prune trees in early spring but not during the remainder of the growing season. This raised questions concerning the stability of the crinkle symptom in affected prune trees.

Consequently, orchards were selected in 1953 for study of the behavior of the condition in individual trees.

MATERIALS AND METHODS

Two orchards were selected for mapping on an individual tree basis. One contained 676 3-year-old Stanley prune trees (Prunus domestica), and the other contained 336 Italian prune trees (P. domestica) ranging from 8 to 15 years of age. Both orchards were inspected in mid-July of 1954-1957. Crinkle symptoms were recorded as present or absent for each tree.

One tree, which had shown crinkle symptoms during the preliminary survey of 1951-1953, was selected in each of seven additional Italian prune orchards for repeated observation during 1959. A different sample of seven trees, each tree in a different orchard, was selected for repeated observation during 1960. Tree age ranged from 3 to 21 years in both cases. These 14 trees were inspected thoroughly during mid-May, mid-July and mid-September; crinkle symptoms were recorded as present or absent.

Budwood was taken from seven additional Italian prune trees in early spring of 1957 and chip-budded to 2-year-old virus-free Italian trees, three buds of each source grafted to each of two acceptor trees. In four of seven cases, donor budwood was taken from twigs which showed prominent crinkle symptoms during the preceding year. The other three samples were taken from orchard trees which showed no symptoms of any kind the previous year.

Virus-free Italian prune buds were transferred to other acceptor trees to serve as controls. Growth from all donor buds was studied during the period 1957-1960.

In all cases, crinkle symptoms were recorded only when both the irregular leaf margins and chlorotic lines regarded as characteristic of the disorder were present.

RESULTS

Total numbers of crinkle-affected trees in the two mapped orchards never were the same on any 2 years of the study (Fig. 1). Incidence of symptoms was low in 1954 and 1957; 1956, com-

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[1] Approved by the Director as Research Paper No. 551; study conducted as a part of Western Region Project W-64.

[2] Associate Plant Pathologist, University of Idaho Agricultural Experiment Station.

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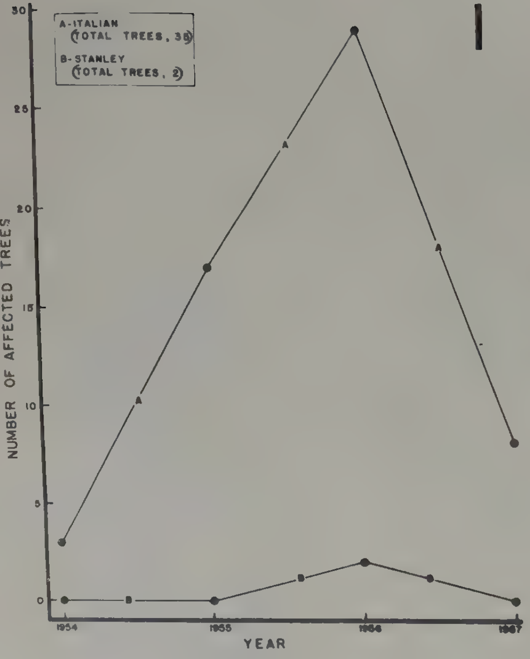

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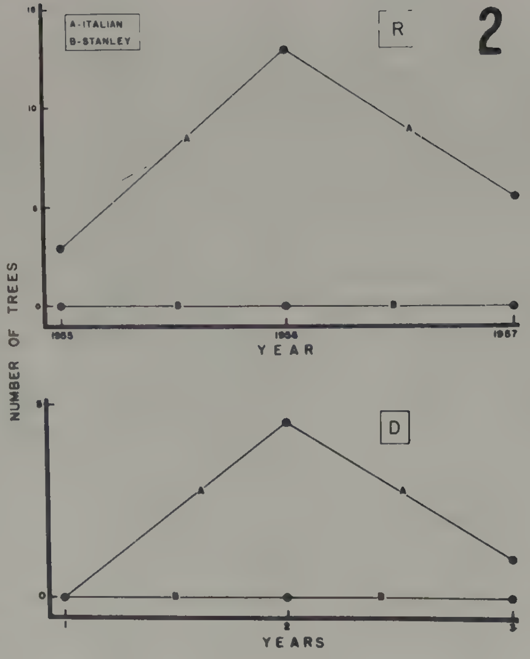

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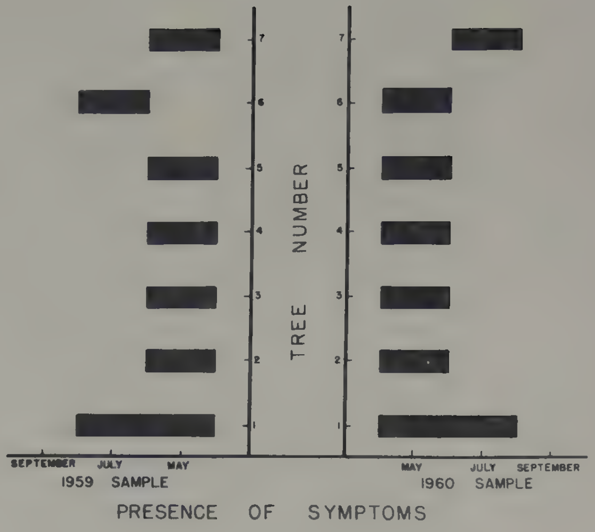

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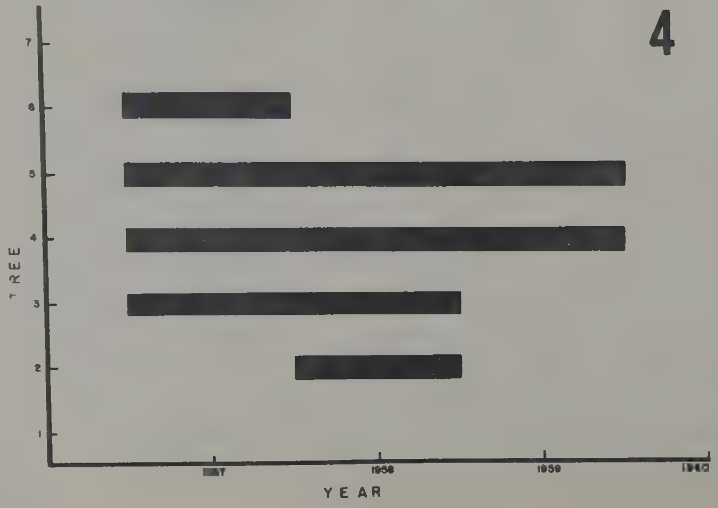

FIGURE 1. Annual incidence of crinkle in an Italian prune orchard of 336 trees and a Stanley prune orchard of 676 trees.

FIGURE 2. Recurrence (R) of crinkle symptoms in previously affected trees in an Italian prune orchard of 336 trees and a Stanley prune orchard of 676 trees and duration (D) of the recurrence in individual trees.

FIGURE 3. Seasonal variation in symptom expression in two groups of selected, crinkle-affected Italian prune trees ranging in age from 3 to 21 years.

FIGURE 4. Manifestation of crinkle symptoms in growth from Italian prune buds taken from crinkle-affected twigs (trees 4-7) and healthy-appearing twigs on unaffected trees (trees 1-3) and grafted to 2-year-old virus-free Italian trees. (Three buds from each source tree chip-budded to each of two acceptor trees in May 1957.)

paratively, was very high. Recurrence in individual trees also varied annually (Fig. 2), but again 1956 was the high point of the 4-year study. Not only was the total number of recurrences in affected trees highest in 1956, there was a sufficiently greater number of 2-year-recurrence cases (that is, affected in 1954-1956) during that period that very similar curves were obtained when total number of recurrences (Fig. 2R) and recurrence-duration in individual trees (Fig. 2D) were plotted against time.

Such annual fluctuations in incidence of crinkle, when observations were made only at one time during the year, led to the seasonal-variation studies of 1959 and 1960 that are illustrated in Figure 3. The data verified the observations of the preliminary survey years in that the selected (mildly affected) trees commonly exhibited symptoms in May, whereas those same trees often showed no symptoms at other times of the year. In none of the 14 cases were symptoms found as late as September.

When budwood was taken from crinkle-affected twigs of four Italian prune trees and budded to virus-free Italian trees, crinkle symptoms developed on donor bud growth in three of the four cases (Fig. 4). Crinkle symptoms also developed in donor-bud growth from buds taken from two of the three other orchard trees which showed no symptoms.

Attempts to bud-perpetuate the crinkle condition in cherries in Idaho have failed more often than they have succeeded, but good results generally have been obtained in prune-crinkle trials. During the budding study described above, many other source trees were tested but are not illustrated in Figure 4. Bud failure as well as apparently healthy bud growth occurred for all of these other sources. This was true whether or not the orchard trees showed symptoms of crinkle or other conditions. Figure 4 therefore illustrates selected cases which demonstrated that buds from crinkle-affected twigs may or may not perpetuate the crinkle condition and that the crinkle condition may arise in bud growth from trees which did not show crinkle symptoms during the growing season preceding collection of the buds.

In all bud-perpetuation studies, a virus-free clone of Italian prune not known to show crinkle symptoms served as the control source. All bud growth from this source was vigorous and free from symptoms.

DISCUSSION AND CONCLUSIONS

Since no causal factor is graft-transmissible with present methods, studies involving the crinkle condition in prunes must rely on visual evaluation. This invites a number of errors which can be guarded against best by maintaining a continuous awareness of them until more precise methods have been developed. In the writer's opinion, classification of early, or transient, symptoms as "false" symptoms is one such error. The mere presence of recognizable, though transient, symptoms is at present more finite evidence that these conditions are a part of the crinkle picture than is their transiency evidence that they are not.

Since the crinkle condition in both prunes and cherries has been under close scrutiny in Idaho since 1951, the writer presumes that the evaluations of this study are as accurate as 10 years of observation will permit.

With this assumption, the study revealed that 1) annual incidence of crinkle symptoms varies with different years; 2) recurrence, and duration of recurrence, in individual trees also varies annually, with only the severe forms being stable; 3) symptom expression in affected trees is not necessarily constant in any one growing season, total incidence being greatest in the spring; 4) budwood from crinkle-affected twigs often, but not always, produces crinkle-affected foliage though such symptoms generally are not indefinitely recurrent in that foliage; and 5) buds from non-crinkle trees may also produce crinkle-affected foliage.

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SUSCEPTIBILITY OF PEACH ROOTSTOCKS TO ROOT-KNOT NEMATODES[1]

G. Minz and E. Cohn

Abstract

Surveys conducted in established peach orchards in Israel revealed that the bitter almond and peach varieties serving as rootstocks are particularly susceptible to *Meloidogyne javanica*. A detailed survey of an experimental orchard with five different rootstocks showed that the peach varieties Shalil, Elberta and Beladi are highly susceptible to this nematode. The Klabi apricot variety is apparently immune. Although the peach variety S-37 showed a fairly high overall index of resistance, a number of trees were found heavily infested, with a high rate of nematode reproduction. This appears to be due to variability for nematode resistance among seedlings of S-37.

INTRODUCTION

The problem of peach rootstocks susceptible to root-knot nematodes has long been a source of concern to growers in Israel. Many peach orchards grown on bitter almond and several peach rootstock varieties have suffered severe damage, incurred both directly and indirectly by nematodes. Several orchards in the northern Negev have had to be uprooted in their seventh year owing to severe decline and death of a large percentage of the trees. Soil fumigation, although often practiced, is not only expensive but has proven relatively ineffective in cases of heavy nematode infestation and severe decline.

Several workers have studied the response of some peach rootstocks to root-knot nematodes. Clayton (4) and Christie and Havis (3) reported that the varieties Shalil and Yunnan, previously considered fairly resistant to root-knot nematodes, were susceptible to some "races" of the nematode. Chitwood, et al. (2) and Havis, et al. (9) consider the S-37 variety resistant to both *Meloidogyne javanica* and *M. incognita*, while Yunnan is susceptible to *M. javanica* but resistant to *M. incognita*. Good (6) refers to the Yunnan and Shalil varieties as being resistant to *M. incognita* and *M. incognita* var. *acrita*, but susceptible to *M. javanica*, whereas S-37 is more resistant to *M. javanica* although it may be invaded to some extent by larvae of this nematode. He mentions that there is, however, very little population buildup, because most of the females which develop fail to produce eggs. This has also been shown by Chitwood, et al. (2). Hansen, et al. (7, 8) and Lownsberry, et al. (10), on the other hand, have shown S-37 to be susceptible to *M. javanica*, although resistant to *M. incognita* var. *acrita*.

It is noteworthy that these data are based mostly on inoculation experiments with young seedlings. The purpose of the present work was to study the susceptibility to root-knot nematodes of some peach rootstocks in established orchards in view of the conflicting conclusions arrived at in previous papers.

PRELIMINARY SURVEYS

Root samples were taken from a number of peach trees in various locations in Israel in order to study the prevalent root-knot nematode species attacking local rootstocks. A total of 24 trees on bitter almond rootstock (*Prunus amygdalus* var. *amara*) and 20 trees on peach rootstock (*P. persica*) were sampled. Posterior cuticular patterns of nematodes from each location represented in the survey were prepared for identification. Results are presented in Table 1.

A second survey was conducted in a peach orchard at Acre, northern Israel, covering a number of rootstocks, known from the literature to be either tolerant or partially resistant to root-knot nematodes. In this survey seven of eight sampled trees on the Yunnan variety, three of six on the Shalil variety and one of three on the S-37 variety were found infested. Infestation of the roots of Yunnan was particularly severe. All three apricot rootstocks (*P. armeniaca*) of the local Klabi variety examined were free from root-knot nematode infestation. A number of rootstocks for plums were also examined in the same orchard. Three Marianna types (2624, 2623, and 4001) and Myrobalan 29 (*P. divaricata*) were free from nematode infestation. Very

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[1] Publication of the National and University Institute of Agriculture, Rehovot, Israel. 1962 Series, No. 489-E.

Table 1. Root-knot nematode species on bitter almond and peach rootstocks in various locations in Israel.

Rootstock	Number of locations	Number of patterns identified: *M. hapla*	*M. incognita*	*M. incognita acrita*	*M. javanica*
1) *Prunus amygdalus* var. *amara*	17	-	1	32	110
2) *P. persica*[a]	10	5	14	10	124

[a] The peach varieties that served as rootstocks could not all be identified, but many of them were known to be of the local Beladi variety and others of two common cultivated varieties, Babcock and C. O. Smith.

Table 2. Infestation by root-knot nematodes of five rootstocks in a peach orchard.

Rootstock	Number of samples examined	Number of samples in each grade of infestation: 0	1	2	3	4	5	Average grade of infestation	Index of resistance[a]
Shalil	32	5	4	4	9	3	7	2.7	46.3
S-37	32	17	4	3	1	4	3	1.4	72.5
Elberta	32	6	8	4	5	3	6	2.3	54.4
Beladi	32	8	2	5	4	6	7	2.6	48.1
Klabi apricot	32	32	0	0	0	0	0	0	100

[a] Obtained from the equation: Index of Resistance = $\left(1 - \frac{\Sigma a}{5\ n}\right)$ x 100 where Σa is the sum of infestation scores, and 5 n is the highest rating value which can be obtained, n representing the number of plants. (Adapted from Bingefors (1).)

few females were recovered from the roots of two trees of the sour sport of *Prunus cerasus* and a single female was detected on roots of one tree of the sweet sport of this variety. Fifty-two patterns were examined from the infested varieties in this orchard (Yunnan, Shalil, S-37, and *P. cerasus*) and all were identified as *M. javanica*. In the rhizosphere of all the trees examined, varying amounts of *Xiphinema* spp. and *Tylenchorhynchus* spp. were identified. In addition to these genera, *Criconemoides* and *Trichodorus* were present in the soil around most of the rootstocks mentioned above.

Following these findings, it was decided to carry out a detailed survey of peach trees on rootstocks which had shown some measure of resistance, particularly on Shalil and S-37 peaches and the Klabi apricot variety.

PROCEDURE

In the summer of 1961, a survey was conducted in an entire 3-year-old experimental orchard, of the Division of Horticulture of this Institute, known to be infested by root-knot nematodes. The orchard contained two peach varieties, Bonita and Ventura, grafted in equal proportions on five different rootstocks: Shalil, S-37, Beladi, Elberta, and the Klabi apricot variety. The orchard is laid out in eight-tree plots of each of the five rootstocks, in four replicates, so that all rootstock varieties are fairly equally distributed throughout the entire area of the grove. Hence, the total number of trees in the orchard is 160, and each rootstock variety occurs 32 times. Sampling of all trees was carried out as follows: roots were removed from three sites of each tree, comprising a composite sample. In each sample both young and older roots were included. The roots were examined in the laboratory and infestation was rated on a 6-scale basis from 0 - no galling to 5 - severe galling.

Egg masses were not counted systematically to establish the rate of reproduction of nematodes, as suggested by other workers (1, 9). This was not felt to be necessary as all samples comprised both young and older roots and this in itself served to indicate that reproduction had occurred. However, the presence of egg masses was always recorded and it was observed that in samples graded 4 and 5, where both young and older roots were heavily infested, egg masses were always abundant. Root samples graded 3 also usually bore egg masses. Roots graded 1 and 2 were usually lacking egg masses, and galls invariably occurred on young roots only. Often no nematodes were recovered from these galls, although on many occasions the galls were fairly large.

Cuticular patterns were prepared from infested roots and identified.

RESULTS

Results of the survey are presented in Table 2.

Four trees which died during the course of the survey and which were found to be heavily infested by nematodes were S-37, Elberta and two trees of Beladi.

Identification of patterns revealed that all four peach rootstocks were infested by M. javanica.

CONCLUSIONS

It seems fairly clear that the predominant nematode species infesting all the peach rootstock varieties throughout Israel is M. javanica; the same is true for the bitter almond rootstock. This is of some interest since an earlier survey of the distribution of root-knot nematodes in this country (11) revealed the widespread prevalence of other root-knot nematodes, in addition to this species, namely M. hapla, M. incognita, M. incognita var. acrita, and a lesser distribution of M. arenaria. It appears, therefore, that most peach varieties, including S-37, are particularly susceptible to M. javanica.

When the relative resistance of the various rootstocks to these nematodes is considered, clearly the three varieties Beladi, Shalil and Elberta could not serve as suitable rootstocks. Klabi apricot is evidently immune to root-knot nematodes. This has also been shown to be the case with other apricot varieties (4, 5). However, horticulturists usually hesitate to accept the apricot as a rootstock for peaches owing to a number of disadvantages in this combination, and prefer to search for a nematode-resistant peach variety.

From the results presented here, the S-37 rootstock showed the highest index of resistance among the peach varieties. It should be emphasized that just over half of the trees on this rootstock examined in the orchard were found to be entirely free from nematode infestation. However, a number of this variety were infested by M. javanica and at least 7 (graded 4 and 5) were found to be heavily infested with a high rate of nematode reproduction. Observations in other parts of the country have shown that some peach trees on S-37 rootstock have considerable field resistance despite heavy infestation with root-knot nematodes. Nevertheless, many trees on this rootstock have suffered severe damage and in the orchard under survey one tree died where M. javanica was found to be the pathogen.

It is noteworthy that Havis, et al. (9), though considering S-37 highly resistant to M. javanica, report that egg masses and females of this nematode were found in one sample of roots from a single plant, and attribute this to "a mistake in labelling or due to genetic differences between plants." From our findings, we tend to believe that the latter is the case. All the varieties studied by us were grown from seeds. It is our view that the conflicting results of experiments with S-37 and other nematode-resistant peach rootstocks stem from the high degree of variability among seedlings of these varieties, rather than from the susceptibility to species of Meloidogyne.

In preliminary inoculation tests we again found considerable variability among seedlings of S-37, and of the Rancho peach variety. We feel that the problem of nematode-resistant peach rootstocks should be solved by vegetative propagation of resistant types of peach varieties, and S-37 could serve to produce suitable clones. Further work in this direction is now under way.

Acknowledgment: We are indebted to Prof. M. R. Samish, Head, Division of Horticulture, The National and University Institute of Agriculture, Rehovot, Israel for placing the experimental orchard at our disposal.

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A REPORT ON THE TESTING OF ONION VARIETIES FOR RESISTANCE TO LESION NEMATODES[1]

G. B. Bergeson[2]

Summary

Of 32 onion varieties tested, none was immune to infection by Pratylenchus penetrans. One variety, Cooper Gem, had a lower nematode population than some of the commonly grown varieties. Wild onion was more resistant than any of the commercial or experimental varieties tested.

Onion yields from several large farms in northern Indiana are severely reduced by the lesion nematode, Pratylenchus penetrans (Cobb). Retarded growth is often apparent shortly after emergence. The nematode enters the delicate sparse root system and begins to reproduce soon after the rootlets are formed. Eggs have been recovered from seedling roots 7 days after seed germination. Initial root lesions are long, narrow, greyish and opaque when compared with the glistening whiteness of uninfected portions. The lesions later become tan and increase in size as reproduction continues. Dissection of new lesions has shown that a single nematode can cause a lesion 1 to 3 mm long and 1 to 2 cells wide.

So far, the only solution to this problem has been the use of soil fumigants. Since this disease is most common in muck soils, the amount of fumigant required for control is double that used in lighter soils. This paper presents the results of an evaluation of selected onion varieties for nematode resistance which, if found, could be used as an alternative to chemical control.

MATERIALS AND METHODS

Naturally infested soil from an onion field was thoroughly mixed and placed in 4-inch pots. Thirty varieties[3], replicated fivefold, were planted at the rate of 10 seeds per pot. Onions grew for 5 weeks in the greenhouse, after which they were processed by carefully washing the roots free of soil, weighing them, and incubating them for a period of 1 week in 100 ml of distilled water receiving continuous aeration. Emerged lesion nematodes were concentrated in 10 ml of water from which two 1-ml samples were withdrawn for counting.

RESULTS

Table 1, test 1, lists the varieties in ascending order of nematodes/gram of root. None of the varieties was immune to the nematode. A few varieties had significantly fewer nematodes than others, but they were not significantly different from the general mean (466/gram of root); neither was there a significant correlation (r = .343) between numbers of nematodes and root weight.

The four varieties with the lowest number of nematodes/gram of root were retested by comparing them with two varieties with high nematode counts. Also included was a previously untested variety, Trapps, and wild onion, Allium canadense. This test was done in the same manner as the first except replications were tenfold and wild onion was grown from bulbs rather than seed. Because of higher greenhouse temperatures, the onion growth was poor and root weights were much less than in the first experiment.

Cooper Gem (Table 1, test 2) was the only one of the four varieties that continued to have an appreciably lower count than some of the other varieties, but again it was not significantly different from the general mean (499/gram of root). The reversal in performance between the first and second test of some of the other varieties, that is, Early Harvest #5 and Downing Yellow Globe, casts considerable doubt on any variety's being more susceptible or resistant than any other variety.

The reduced nematode count in the roots of wild onion was significantly different (1% level)

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[1] Journal Paper No. 1927, Purdue Agricultural Experiment Station, Lafayette.

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[3] Seeds of the varieties tested were kindly furnished by C. I. Harris of the Purdue Horticulture Department.

Table 1. Root weight and number of Pratylenchus penetrans per gram of root in 32 onion varieties.

Variety	Source[d]	Test 1		Test 2	
		root weight (grams[a])	nemas/gram[a]	root weight (grams[b])	nemas/gram[b]
Wild onion				.47	77
Early Harvest # 5	1	3.1	259	.23	640
Cooper Gem	2	3.5	292	.65	234
Downing Yellow Globe	3	2.7	292	.45	792
Early Harvest	2	2.6	304	.39	422
EH[c] 9P/249	3	4.2	340		
XP 2229	2	2.7	353		
Pilot	4	3.5	366		
Golden Beauty	2	3.4	383		
EH9P/234	3	2.8	402		
Treasure	4	2.9	413		
XP 2233	2	2.4	424		
Brown Beauty	3	2.9	441		
EH9P/242	3	3.2	448		
EH9P/238	3	3.7	460		
Magnifico	3	2.6	468		
Bronze Perfection	2	3.3	474		
Encore	4	2.5	484		
EH9P/141	3	2.1	491		
Abundance	4	3.0	503		
Surprise	2	2.3	518		
Early Yellow Globe	4	2.8	530		
Autumn Splendor	3	2.1	541		
Fiesta	4	3.6	541		
EH9P/244	3	4.9	546		
EH9P/250	3	1.7	590		
Premier	4	2.3	603		
Autumn Topper	3	2.2	616		
Autumn Spice	3	2.5	638		
Aristocrat	4	2.1	640	.62	646
Empire	4	1.5	651	.59	527
Trapps				.49	654
LSD 5%			312		413
1%			419		544

[a] average of 5 replications.
[b] average of 10 replications.
[c] EH = Experimental hybrid.
[d] 1 - Ferry Morse Seed Co. 2 - Northrup King Seed Co. 3 - Crookham Seed Co. 4 - USDA.

from five of the seven varieties and significantly different (5% level) from the general mean. Wild onion was retested in the same manner in a tenfold replicated experiment that included the variety Empire for comparison. The initial soil population in this experiment was intentionally lowered to increase the chance that nematodes eventually recovered from the roots would represent new progeny rather than members of the original population. After a 6-week growing period, the nematodes/gram of root for wild onion were 12.8 compared with 109.9 for Empire. Furthermore, lesions on wild onion roots appeared to be more woody and smaller than those on commercial varieties. Also, the nematode colonies within the lesions had fewer eggs and the spread of the nematodes within the root appeared to be restricted by the heavy-walled cells surrounding the lesion.

In conclusion, these evaluations failed to find a commercial variety that could be considered resistant enough to be used as an alternative to chemical control. The most promising source of resistance was found in wild onion. The full nature of this resistance will be further investigated.

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REACTION OF BEANS TO TOMATO AND TOBACCO STRAINS OF TOBACCO MOSAIC VIRUS[1]

E. M. Johnson

Summary

Eleven strains of TMV from tomatoes in plastic greenhouses, three strains of TMV from Pennsylvania that cause internal browning of tomato and several strains of Plantago virus maintained in either tobacco or tomato did not induce necrotic spotting of Pinto and Golden Cluster Wax beans, whereas the TMV from tobacco used in this study regularly caused necrotic spotting on these bean varieties. The tomato strains of TMV, the virus strains from Pennsylvania, Plantago virus and TMV from tobacco could not be distinguished from each other by heat treatment or dilution tests. It is suggested that the tomato strains of TMV may be intermediate between Plantago virus and TMV.

INTRODUCTION

In studies being made on the effect of moist heat on the inactivation of TMV in cured burley tobacco, Pinto and Golden Cluster Wax beans were at first used as assay plants because TMV usually induces necrotic spots on these plants. A strain of TMV from tomato failed to induce necrotic spotting on these hosts. Therefore, a number of strains of TMV from tomato and 20 strains of Plantago virus from *Plantago rugellii* and *P. lanceolata* also known not to induce necrotic spotting on beans (1) were collected for comparative studies with TMV from tobacco.

MATERIALS AND METHODS

Whenever virus-affected tomato plants were received for disease identification, inoculations were made to mosaic susceptible Ky 16 (nn) burley tobacco plants (*Nicotiana tabacum*) and these were saved for study. Eleven tomato strains of TMV were isolated from commercial tomato crops grown in plastic greenhouses in widely scattered geographical areas in Kentucky. These and three tomato strains of TMV from Pennsylvania designated CIB, CM 316, and M97 that were sent by Dr. J. S. Boyle to Dr. D. J. Cotter of the Horticulture Department, University of Kentucky were used in these studies. Also used was a tobacco strain of TMV originally collected in 1927 and 20 strains of Plantago virus including one preserved in dried tobacco since 1940.

Unifoliate leaves of Pinto and Golden Cluster Wax beans dusted with 600 mesh carborundum were rubbed with autoclaved glass spatulas dipped in fresh viruliferous tissue crushed in m/100 dipotassium phosphate. Immediately following inoculation the leaves were washed with tap water. Infected tissue of both tomato and tobacco were used for inoculations. Whether the inoculum came from tomato or tobacco appeared not to matter because the results were the same. Therefore, infected tobacco tissue was used in most of the studies because tobacco plants are easier to maintain in the greenhouse for long periods.

RESULTS

The tomato strains of TMV, including those from Pennsylvania, the tobacco strain of TMV, and the Plantago viruses could not readily be distinguished from each other on Bonny Best and Marglobe tomato (*Lycopersicon esculentum*) and mosaic susceptible Ky 16 (nn) burley tobacco where all caused mottling, slight distortion and stunting.

The tomato viruses, Plantago virus and TMV from tobacco caused necrotic spotting on Ky 35 (NN) burley tobacco, on *N. glutinosa*, on Black Beauty egg plant (*Solanum melongena* var. *esculentum*), and on Lipstick petunia (*Petunia hybrida*). None of these plants developed mottling.

When *P. rugellii* plants were inoculated with the viruses listed in Table 1, only those inoculated with Plantago virus developed systemic mottling.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

Table 1. Reaction of Pinto and Golden Cluster Wax beans to tomato, tobacco, and Plantago viruses.

Virus number	Source		Pinto		Golden Cluster Wax	
			Plants inoculated	Necrotic spots on	Plants inoculated	Necrotic spots on
1	Tomato	Kentucky	16	0	12	0
2	do.	do.	44	0	44	0
3	do.	do.	8	0	8	0
4	do.	do.	4	0	4	0
5	do.	do.	4	0	4	0
6	do.	do.	28	0	28	0
7	do.	do.	8	0	8	0
8	do.	do.	16	0	16	0
9	do.	do.	16	0	16	0
10	do.	do.	64	0	64	0
11	do.	do.	44	0	28	0
12	do.	Pennsylvania	16	0	16	0
13	do.	do.	16	0	16	0
14	do.	do.	16	0	16	0
15	Plantago	Kentucky	44	0	28	0
16	Tobacco	Kentucky	24	24	20	20

In Table 1 the combined results of inoculations to Pinto and Golden Cluster Wax beans made over a period of several months are given. Eleven strains of TMV from tomatoes from plastic greenhouses and three Pennsylvania strains of TMV did not induce necrotic spots on unifoliate leaves of Pinto and Golden Cluster Wax beans. Twenty strains of Plantago virus also did not induce necrotic spots on these bean varieties, whereas TMV from tobacco regularly induced numerous necrotic spots on them.

In another test the left sides of unifoliate leaves of Pinto and Golden Cluster Wax beans were inoculated either with the tomato strains of TMV, the Pennsylvania viruses, or with Plantago virus and the right sides of the same leaves were inoculated with TMV from tobacco. In this test the left sides of all inoculated leaves were free from necrotic spots whereas necrotic spots were numerous on the right sides of all inoculated leaves.

PHYSICAL PROPERTIES OF THE VIRUSES

Expressed fresh crude juice from tobacco inoculated with the viruses in Table 1 was variously diluted with a 2 to 1 mixture of M/10 dipotassium phosphate and M/10 monopotassium phosphate and inoculations were made to NN Ky 35. All viruses had slight activity at a dilution of 10^{-7} but none at 10^{-8}. With undiluted juice, an inoculated leaf of Ky 35 developed about 1500 necrotic spots. At a dilution of 10^{-7}, the number of necrotic spots on inoculated leaves was reduced to 2 to 5.

Two ml of expressed crude sap of all viruses used in this study, diluted with distilled water to 10^{-2}, were subjected in thin-walled pyrex glass tubes (12 x 110 mm) to temperatures varying from 70° to 90° C for 10 minutes. At the end of the heat treatment the tubes were immediately plunged into ice water. Inoculations were made to carborundum-dusted leaves of Ky 35. All viruses survived a temperature of 87.5° for 10 minutes but all were inactivated at 90° for 10 minutes.

DISCUSSION

Miller and Thornberry (3) isolated a virus from tomato and later from pepper which they describe as atypical mosaic virus (TAMV). This atypical virus, like the tomato strains of TMV in this study, withstands a dilution of 10^{-7} and does not induce necrotic spots on inoculated leaves of Golden Cluster Wax and Pinto beans. Miller's and Thornberry's TAMV however is inactivated at 72° C for 10 minutes, whereas the infectivity of the tomato strains of TMV is not destroyed at 87.5° for 10 minutes.

Valleau (4) suggests that the strains of TMV are mutants of the Plantago virus and that TMV actually had its origin when the Plantago virus became established in cultivated tobacco rather than that the Plantago virus originated from the tobacco mosaic virus as suggested by Holmes (2). Possibly the tomato strains of TMV might be considered as transitional between Plantago virus and the common strains of TMV found in tobacco. Little alteration of the nucleic acid might be necessary to effect the change from Plantago virus to TMV.

Certain bean varieties, including those used in this study, have been used in studies to determine the presence of TMV in fresh and dried tissue. That this method is unsafe is shown by the fact that at least some strains of TMV from tomato and at least one strain from pepper (3) fail to induce necrotic spots on bean varieties susceptible to necrotic spotting by most tobacco strains of TMV. Many more strains of TMV incapable of causing necrotic spotting of certain bean varieties may be present in weed hosts. N. glutinosa or necrotic spotting varieties of tobacco such as Ky 35 or Burley 21 rather than bean varieties should be used in studies to determine the presence of TMV in plant material. The necrotic spots on N. glutinosa and necrotic spotting varieties of N. tabacum are more conspicuous than those caused by TMV on susceptible bean varieties and so far all strains of TMV tested have induced necrotic spots on N. glutinosa and mosaic resistant tobacco varieties.

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KENTUCKY AGRICULTURAL EXPERIMENT STATION, LEXINGTON

PYTHIUM DAMPING OFF IN TOBACCO PLANT BEDS

E. M. Johnson

Damping off caused by Pythium sp. has appeared in many tobacco plant beds in Kentucky. This disease is extremely rare, having possibly occurred several years ago in one or a few beds. Rotting begins on the stems at the soil surface and extends up the stem into the leaf petioles, causing the leaves to appear scalded. The disease usually appears in isolated areas in beds. Some growers, and a few county agents, have mistaken this disease for blue mold. During the appearance of the disease, the temperatures have been 90° F and above which is unusually high for this season.

KENTUCKY AGRICULTURAL EXPERIMENT STATION, LEXINGTON

STERILIZATION OF WHEAT SEED BY ETHYLENE OXIDE

James F. Schoen[1]

Wheat from countries listed in Federal Quarantine 319.59 (Flag Smut) is prohibited entry into the United States except under Departmental Permit. When small lots are entered under such permits for experimental baking, milling, or weed seed separation tests, there is a need for a satisfactory means of sterilizing the grain by a method that will not affect qualities essential to such testing.

Ethylene oxide, which is used commercially to fumigate spices, is a highly effective sterilant. A non-flammable mixture containing 12% by weight of ethylene oxide in Freon 11 and Freon 12 gases was found convenient and safe to use at this Station. This mixture is readily available commercially.

To test the efficacy of the gas, 25-pound lots of wheat in cloth sacks were separately exposed overnight in a large polyethylene bag at temperatures of 28° to 30° C. Small samples of the grain taken from various depths within the seed lots gave consistently negative results when culturing was attempted by placing them in AC broth and Sabouraud liquid media. This was in contrast with strong positive results when untreated seed was cultured.

The efficacy of ethylene oxide in sterilizing a fairly large quantity of wheat within a 93 cubic foot chamber was shown by a treatment given to six sacks of wheat each weighing 120 pounds. The dosage was approximately 300 mg per liter at room temperature for 64 hours. A vacuum of 20 inches was drawn prior to injection of the gas but after its entrainment the vacuum was reduced to approximately 3 inches.

Ten seed samples of 2 grams each (five samples from each of two sacks) were taken and divided equally for culturing in both AC broth and Sabouraud liquid media. All samples proved negative for organisms. Germination tests made immediately after treatment showed that the seed was completely non-viable as compared with 92% germination of untreated checks.

Five-pound samples of three types of wheat, fumigated with approximately 560 mg per liter of space at 26° to 28° C for 18 hours, were given milling and baking tests by the Grain Division, Agricultural Marketing Service, United States Department of Agriculture.

When compared with the control, the dough from the flour of the fumigated soft winter wheat had an off odor, the odor and taste of the bread were also off, the crumb score was reduced and the loaf volume dropped by more than 100 ml. In the case of hard winter wheat the odor of the dough was slightly off but the bread odor, taste, and loaf volume were unaffected although the crumb score was reduced. Fumigation of hard spring wheat produced no change in bread loaf volume but the crumb score was reduced and the odor of both the dough and bread was slightly off.

In view of the milling and baking tests, it appears that ethylene oxide is unsuitable for sterilization of wheat intended for use as flour for baking.

PLANT INSPECTION STATION, PLANT QUARANTINE DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

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FIRST RECORD OF HOP CYST NEMATODE (HETERODERA HUMULI FILIPJEV, 1934) IN THE UNITED STATES

Grace S. Cobb[1]

In March 1962 nematode cysts collected from soil with hop cuttings (Humulus lupulus) from Canada were sent in for identification. This material was intercepted and processed by William J. N. Brown and Sueo Nakahara, of the Plant Quarantine Division, Seattle, Washington. Although the cysts appeared to be Heterodera humuli Filipjev, 1934 they were in too poor condition for positive identification. It was therefore suggested that collections be made in some nearby hop-growing areas to see whether the nematode occurred in the United States and to secure better specimens for study. Accordingly, in April 1962 Dr. Brown and Mr. Nakahara made a number of collections on hop farms at Orting and Roy, Pierce County, Washington. Cysts from these soil samples, as well as some from the roots of growing hop plants, proved to be Heterodera humuli; they represent the first record of this nematode in the United States.

The hop cyst nematode, long known to occur on hops in Germany and England, is not considered as the cause of any particular disease of that host. In the United States this nematode has been heretofore identified only in foreign Plant Quarantine interceptions of hop material from Switzerland in 1955, England in 1957, Germany in 1958, and possibly Yugoslavia in 1959.

Since there is no evidence to indicate the age of the infestations, it is possible that the hop cyst nematode may have existed, undetected, in the hop-growing areas of the Northwest for many years.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

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THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

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Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

29

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Paul R. Miller

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CONTENTS

1. Necrotic shock -- A virus disease of strawberries
N. W. FRAZIER, et al. 547

2. Bacterial leaf spot disease of poinsettia in Florida
LORNE A. McFADDEN and HOWARD R. MOREY 551

3. Isolation and identification of tomato ring spot virus associated with rose plants and rose mosaic virus
ROBERT S. HALLIWELL and J. A. MILBRATH 555

4. Resistance of alfalfa to two species of root-knot nematodes
JOHN H. O'BANNON and HAROLD W. REYNOLDS 558

5. The comparative effects of various fungicide programs on fruit numbers and yields of apple trees
H. F. WINTER 560

6. Tobacco-etch virus on peppers and tomatoes in the Lower Rio Grande Valley of Texas
D. M. McLEAN 565

7. Isolation of a mutant strain of potato Y virus
A. A. COOK 569

8. A pine disease in New Zealand resembling littleleaf
GEORGE H. HEPTING and F. J. NEWHOOK 570

9. Species of root-lesion nematodes commonly associated with economic crops in the Delta of the U. A. R.
BAKIR A. OTEIFA 572

10. Sporulation, germination, and early growth of Echinodontium tinctorium
ORSON K. MILLER, Jr. 576

11. Resistance to yellow dwarf of oats in Washington
G. W. BRUEHL, et al. 579

12. The incidence of necrotic ring spot and sour cherry yellows viruses in commercial mazzard and mahaleb cherry rootstocks
R. M. GILMER and L. R. KAMALSKY 583

13. A survey of plant-parasitic nematodes in strawberry fields of Nova Scotia
MUHAMMED WASEEM and DONALD L. CRAIG 586

14. Anthracnose and powdery mildew on strawberry plants in Louisiana
N. L. HORN and R. G. CARVER 591

15. In vitro effects of seventeen chemical agents on Cytospora cincta
A. E. HARVEY and A. W. HELTON 593

16. Alfalfa seedling losses at varying times after planting
A. F. SCHMITTHENNER and J. L. PARSONS 597

17. New soil fungicides for potato scab and Rhizoctonia control
R. C. CETAS and E. D. JONES 601

18. A method for determining the oxygen requirement of fungi in liquid media
L. J. KLOTZ, et al. 606

19. Summary of reduction in yield caused by the major cotton diseases in the United States from 1957 to 1961
COTTON DISEASE COUNCIL 609

20. Preliminary studies on the control of nematodes in outdoor rose plantings
A. F. SCHINDLER and T. J. HENNEBERRY 610

21. Effect of volume and variation in droplet size of spray applications from an air-carrier sprayer on control of three almond diseases
JOSEPH M. OGAWA and WESLEY E. YATES 614

22. Use of nylon mesh in studies of soil fungi
K. M. OLD and T. H. NICOLSON 616

23. A report of the occurrence of camellia flower blight in Mississippi
J. H. COCHRAN 617

24. The spread of lima bean downy mildew (Phytophthora phaseoli) strain "B" in New Jersey
R. E. WESTER and TEJPAL S. GILL 617

NECROTIC SHOCK -- A VIRUS DISEASE OF STRAWBERRIES

N. W. Frazier, P. S. Jorgensen, H. E. Thomas, and H. A. Johnson, Jr.[1]

Abstract

Necrotic shock is a graft-transmissible virus disease of strawberries that causes temporary symptoms which, in the indicator host F. vesca var. Alpine, include a blackish necrosis of leaves and petioles during the shock stage of infection. Results of mechanical inoculation tests and tests to determine a natural vector have been negative. The disease actively spreads in all the major strawberry fruiting and nursery areas of northern California. Everbearing varieties appear to be more susceptible to natural infection than spring varieties, perhaps indicating a relationship between the habit of flowering and infection.

Necrotic shock virus was first found in strawberry by Jorgensen in 1956 (3). His original isolations were from several numbered strawberry clones which had been grown from seed in a nursery near Modesto in the San Joaquin Valley. Since then, active spread of the virus has occurred also in the Central Coast strawberry fruiting region and in the northern California nursery area, even in a seedling plot in a mountain location isolated by many miles from the nearest strawberry planting.

SYMPTOMS

Symptoms of infection by necrotic shock virus have not been detected on field-grown commercial strawberry varieties. In experimentally infected varieties symptoms consisting of a transient stunting and mild epinasty of leaflets have been observed only on a single leaf of a plant. Subsequent leaves appeared symptomless. The virus persists in, and may be readily recovered by grafting from, vegetatively propagated progeny of an infected mother plant.

The indicator varieties of Fragaria vesca are much more susceptible to injury. In the glasshouse the most sensitive has been the Alpine variety on which the following sequence of symptoms is characteristic:

First, the petiole or leaflets of maturing leaves develop a mild, usually temporary, epinastic curvature. This is followed by blackish necrotic spots or streaks frequently first noticeable at the base of main veins or on the petiole (Fig. 1, A,D). The necrosis usually spreads to kill affected leaves. In younger leaves a mild interveinal chlorosis may develop. Next, very young leaves become stunted and epinastic, and a leaflet or the entire leaf may be killed before unfolding (Fig. 1, B,C,E). This is followed by development of side crowns, at first with dwarfed leaves and red petioles but later with growth becoming more nearly normal. Finally, the new growth appears entirely normal.

On runnering F. vesca clones such as UC-1, UC-3 and the East Malling clone, the symptoms run a similar course but are less severe; there is little necrosis, more chlorosis, and more rapid recovery to an appearance indistinguishable from normal. During onset of symptoms, stolons may develop a temporary somewhat corkscrew appearance with one to several S-curves formed.

Among different isolates of the virus there is considerable variation in the severity of the symptoms produced. The most severe may kill the crowns of Alpine plants and yet they have never been completely lethal for such plants have always developed new crowns and recovered. Cross protection tests have thus far indicated that the isolates protect against one another.

Under field conditions it has not been possible to diagnose necrotic shock infection on Alpine plants with confidence. To prove infection they must be indexed in the glasshouse.

As already indicated, after a relatively short period of symptom expression, infected strawberries recover and the virus becomes latent. Recovered F. vesca plants have been additionally infected experimentally with strains of strawberry mottle, veinbanding, mild yellow edge and crinkle viruses. The effect of the double infections was mildly additive in each case, particularly in the combination with veinbanding virus. It is not known whether this

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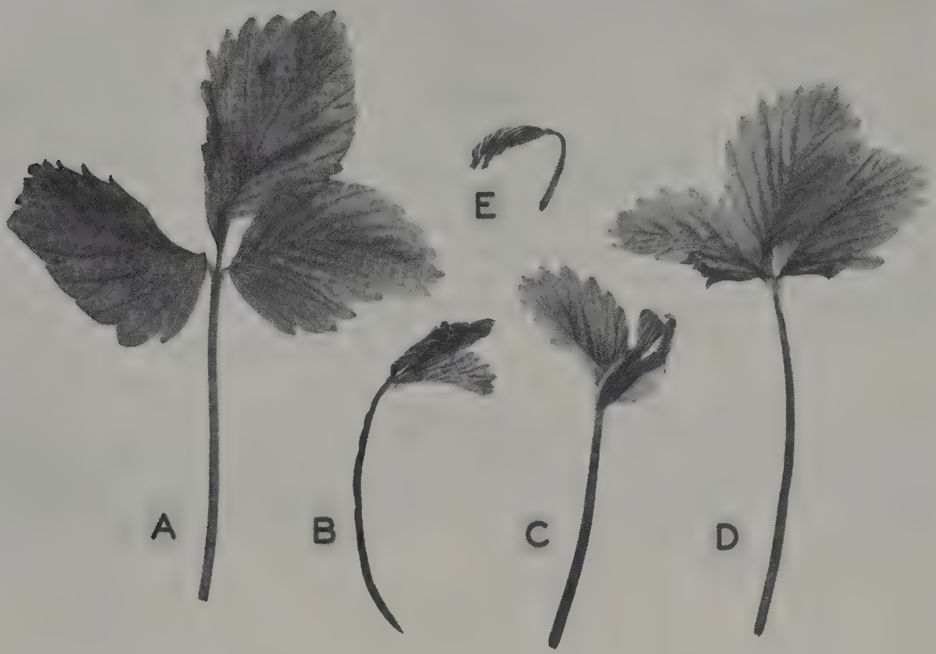

FIGURE 1. Leaves of Fragaria vesca var. Alpine in different stages of maturity from the same plant showing the blackish necrosis symptom of infection by necrotic shock virus.

slight additive effect might be detected in commercial varieties or if it might be deleterious under field conditions.

VIRUS TRANSMISSION TESTS

The necrotic shock virus does not appear to be sap-transmissible; numerous tests which combined variations in technique, virus sources and a range of widely used test plants yielded no instance of virus transmission.

No vector has been found over a 5-year period of testing. Insects of all kinds found on infected clonal plants in the field have been transferred directly to healthy test plants; insects have been collected in and around plantings in which the virus was known to be actively spreading and either placed directly on test plants or first given an acquisition feeding period on diseased plants; attempts to colonize insects on diseased plants were made; the strawberry aphid was tested in particular; and, soil from around diseased field plants has been tested for soil transmission. No instance of infection resulted.

Thus far the virus has been experimentally transmitted only by graft. It is one of the easiest of the strawberry viruses to transmit by this method; and after grafting, symptoms commonly develop in as few as 10 to 14 days, which is a shorter period than is usual with other strawberry viruses.

FIELD SPREAD OF VIRUS

Information was obtained on where the virus was actively spreading, the incidence of such spread, and the season during which it took place. Data could be obtained only by graft-indexing random field plants, since under field conditions no useful symptoms of the virus are expressed.

In this manner, infection of previously healthy plants has been detected in the San Joaquin Valley, the Central Coast fruiting area (where in one instance several plants of a number growing under a screen cage became infected), and during the first season in each of five isolated nurseries in the Red Bluff-Redding-McArthur region of northern California where much of the strawberry nursery stock is produced.

In all localities studied, both spring fruiting and everbearing types of commercial strawberries were grown and after several seasons it became evident that most infected plants were everbearers while relatively few were of the spring type. The plant most susceptible to field infection appeared to be the Alpine indicator variety, which is a runnerless everbearing plant that flowers over most of the growing season. In one seedling plot all of 60 Alpine plants became infected during one season but only about 20% of the commercial octoploid seedling plants. Although the two most widely grown California varieties, Shasta and Lassen, were grown in most of the nurseries with the everbearer varieties, no infection in either variety has been found. Thus, it would appear that infection by the necrotic shock virus is in some way related to the variations in fruiting habits of strawberries.

To obtain evidence on the season of spread, Alpine plants were set out in late September of 1959 in the seedling plot where a high incidence of infection had occurred the previous year. Twelve plants were indexed on the following February 2 and 12 more on May 27 with no evidence of virus transmission, but on July 27 two of five indexed Alpine plants yielded necrotic shock virus and on September 24 two additional plants of eight indexed had become infected.

Another group of Alpine seedlings were planted in the same nursery and also in a second nursery in May of 1960. On July 27 nine plants in the first and eleven in the second nursery were indexed and found virus free. On September 24 sixteen plants in the first nursery indexed virus free but 3 of 12 plants in the second nursery yielded necrotic shock virus. At best these results merely indicate that in the locality of the tests spread of the virus might occur at any time during the 5 summer months of May through September. On the other hand, since new infections were detected only on July 27 and September 24, it is conceivable that the spread could have occurred during a more limited period.

The plants set out in September were well established the following spring and produced a considerable amount of bloom through the season, while those set out in May were not well established and did not produce significant amounts of bloom until the end of July. That the earliest infections were recorded from the older plants tends to support an hypothesis relating the fruiting habit with infection.

A second attempt was made to determine more accurately the period of virus spread by utilizing a different method during 1961: young Alpine seedling plants in 4-inch clay pots were set out in nurseries in the Redding-McArthur area for 2 weeks, replaced with another set of potted test plants and brought to the glasshouse at Morgan Hill to observe for development of virus symptoms. In this manner 10 plants were exposed in each of three nurseries at 2-week intervals from May 15 to October 20. Of a total of 330 plants exposed only 3 became infected. One plant had been exposed from June 2 to 17, the second from June 17 to July 2, and the third from July 18 to August 3, again indicating mid-summer as the season of virus spread in that area. Since no indicator plants were exposed for the entire growing season of 1961 there is no way of knowing whether mature, well established indicators would have become infected in the same location where plants exposed at 2-week intervals failed. One might surmise, however, that the shock sustained by tender, lush-growing indicators supporting some blossoms and fruit when removed from the screenhouse and placed in the field might make such plants less attractive to an assumed vector than would established old plants supporting good flowers and fruit.

DISCUSSION

The necrotic shock virus does not appear by itself to be of economic importance in strawberries because, after infected plants recover from the relatively short period of symptom expression, the virus is carried in a latent state. It is of considerable concern, however, not alone because it does exert a mildly additive influence in combination with other viruses in *F. vesca* varieties, and therefore possibly also in commercial plants, but primarily because it is highly desirable to maintain strawberry nursery stock as free from virus diseases as possible. Because necrotic shock virus spreads in nursery areas as well as in fruiting areas, it is important to determine how it is spread and whether it can be controlled.

Since the virus is latent in strawberry, it is difficult to obtain meaningful amounts of data. Past experience would seem to indicate: 1) that both the virus and its vector are widely endemic in the interior valley, mountain, and coastal regions of northern California; 2) that both the virus and its vector have host plants other than *Fragaria*; 3) that susceptibility to infection is related to the flowering habit of strawberry varieties and, therefore, points to the probability of a flower-visiting vector; and 4) that strawberry may be an unsuitable test plant for vector testing purposes. Under field conditions strawberry is susceptible to infection with the aster yellows virus, but under experimental conditions infections are very difficult to achieve by means of infective leafhopper vectors although other hosts are readily infected by the same insects (1, 2). A similar relationship may exist in respect to infection with the necrotic shock virus.

The symptoms of necrotic shock in F. vesca indicator varieties appear to closely resemble those described by Stace-Smith and Mellor (4) for Rubus yellow-net virus in the same hosts. Rubus yellow-net was ultimately lethal to most infected plants, whereas plants infected with necrotic shock ultimately recover, but this variation could be due to strain differences. According to Stace-Smith and Mellor, the natural vector of the virus, Amphorophora rubi, was not able to infect strawberry, but it could recover virus from diseased strawberries. During the course of the present investigation no Amphorophora aphids were observed on strawberries, but this does not preclude the possibility that individual aphids from Rubus hosts may pass through strawberry plantings and temporarily feed on plants but not remain to colonize. Further work is necessary to either clearly relate or to distinguish between the necrotic shock and Rubus yellow-net viruses.

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BACTERIAL LEAF SPOT DISEASE OF POINSETTIA IN FLORIDA[1]

Lorne A. McFadden and Howard R. Morey[2]

Summary

A disease of Euphorbia pulcherrima, characterized by small, water-soaked, brownish, spots was first observed in Florida in 1960. A bacterium was consistently isolated from diseased lesions and later proved pathogenic to several varieties of poinsettia. Cultural, physiological, morphological and cross-infection tests show the bacterium to be similar to Xanthomonas poinsettiaecola Patel, et al., the cause of a leaf spot disease of poinsettia reported in India in 1951. This is believed to be the first report of the disease in the United States.

INTRODUCTION

A leaf spot disease of Euphorbia pulcherrima was observed in two nurseries in South Florida in 1960. Symptoms of the disease were unlike those previously described on poinsettia in the United States. Its distribution is not believed widespread in the area, but when introduced into a nursery the disease spreads rapidly and may cause extensive leaf spotting and defoliation on susceptible varieties. Previously the diseases most commonly affecting poinsettia in southern Florida were bacterial blight (Corynebacterium poinsettiae Starr & Pirone) (4) and poinsettia scab (Sphaceloma poinsettiae Jenkins & Ruehle) (3). Rogers (7) in 1959 reported a soft rot decay of poinsettia cuttings in Missouri caused by Erwinia carotovora (Jones) Holland. Patel, et al. (5) in 1951 described three new bacterial pathogens from Bombay, India, one of which caused a leaf spot of E. pulcherrima. A study was undertaken to determine the specific identity of the leaf spot pathogen from Florida and to compare it with the one described in India.

DISEASE SYMPTOMS

On the under leaf surface, new spots are dull grey to brown and slightly hydrotic. The spots later become chocolate-brown to rust colored, measure 1 to 2 mm in diameter, and are visible on both surfaces. Lesions vary from more or less circular to angular in shape and on some varieties of poinsettia may be surrounded by a pale green yellow halo (Fig. 1). Spots often coalesce to form large, irregular, necrotic areas which at times extend into the lateral veins causing a blighted appearance. The lesions are often more numerous along the veins and midribs, although not confined to these areas. As disease severity increases, the leaves of certain varieties turn yellow and drop prematurely. Highly susceptible varieties of poinsettia may be almost devoid of leaves at time of flowering. No spots have been observed on the colored bracts. A bacterial exudate often forms on lesions, especially on the lower leaf surface. The ooze dries to form a shiny scale-like crust. Disease spread is correlated with heavy and frequent rains of the spring, summer and fall months in south Florida.

To date natural infections have been observed on several poinsettia varieties including 'Ecke's Flaming Sphere,' 'Ecke White,' 'Improved Henriette Ecke,' 'Henriette Ecke Double, 'Indianapolis Red,' and 'Barbara Ecke Supreme.' Thus, most of the important varieties currently in commercial trade appear susceptible to some degree. The varieties 'Ecke's Flaming Sphere,' 'Ecke White' and 'Henriette Ecke Double' were highly susceptible, while 'Indianapolis Red' proved moderately resistant when artificially inoculated.

ISOLATION AND INOCULATION

A pale lemon-green, mucoid bacterium was readily and consistently isolated from leaf spot lesions that were surface sterilized in sodium hypochlorite and plated directly on freshly prepared PDA, or when leaf lesions were triturated in sterile water and the resulting suspension streaked on PDA or Difco nutrient agar.

Individual colonies of the bacterium were obtained by shaking small loopfuls of the bacterium from PDA slants in 0.1% Sparkleen detergent solution before streaking out on PDA and selecting isolated colonies after 24 to 36 hours.

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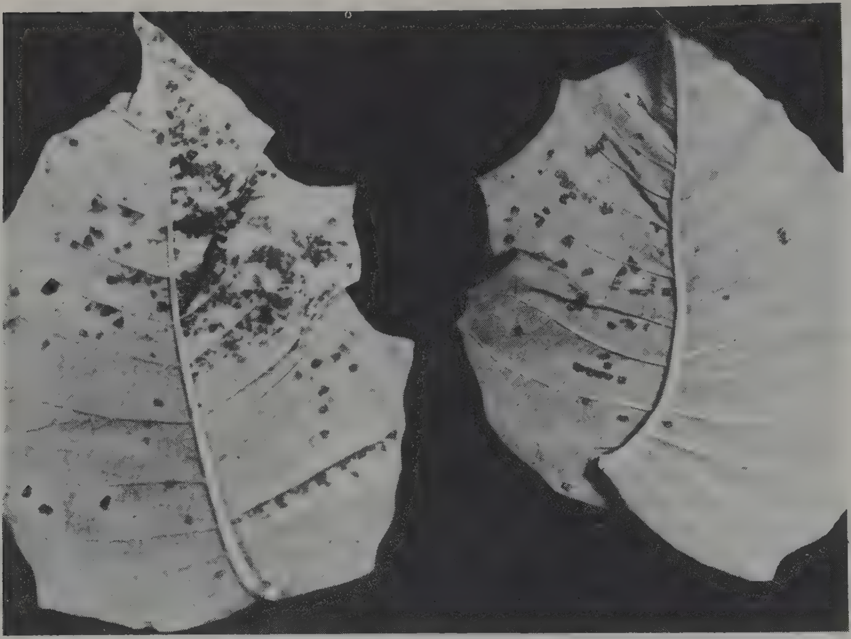

FIGURE 1. Undersurface of leaves of 'Ecke's Flaming Sphere' poinsettia showing natural bacterial leaf spot infection.

Proof of pathogenicity was established by swabbing leaves of 'Barbara Ecke Supreme' poinsettias, previously dusted with carborundum, with 24-hour cultures of the bacterium diluted in sterile water. In 1 week the inoculated leaves produced small water-soaked spots indistinguishable from natural infections, while the control plants remained unchanged. The same lemon-green mucoid bacterium was readily isolated from the inoculated plants.

THE PATHOGEN

Six isolates of equal pathogenicity were selected to study cell morphology, physiological reactions, and cultural characteristics. The methods followed were essentially those outlined elsewhere (1, 6, 8). In the fermentation studies, the carbohydrates and organic acids were filter sterilized and utilized at 1% and 5% concentration respectively, unless stated otherwise. A basal medium containing the following formulae was used in the fermentation tests:

$NH_4H_2PO_4$	1.0 g	
Buffer solution	2.5 ml	(K_2PO_4 7.0 g; KH_2PO_4 3.0 g; distilled water 100 ml)
Trace elements	1.0 ml	($MgCl_2 \cdot 6H_2O$ 10.0 g; $MnSO_4$ 1.0 g; $FeCl_3$ 1.0 g; HCl 1.0 ml; distilled H_2O 100 ml)

Two cultures of Xanthomonas poinsettiaecola Patel, et al., one from ATCC (11643) and another from the National Collection of Plant Pathogenic Bacteria in England (a486) were used for comparison with the pathogen under study.

Morphology: The bacterium was a short rod measuring 1.5 x .60μ (1.1 - 2.2 x .33 - 90 μ) when stained using Congo Red and acid alcohol. The pathogen was gram-negative, occurring singly and in short chains. The cells were encapsulated, and exhibited a single polar flagellum. All isolates were non-motile in Difco-motility medium.

Cultural Characteristics: Growth on freshly prepared potato-dextrose agar was mucoid, luxuriant, and pale greenish-yellow after 24 hours, becoming slightly darker on longer incubation. On Difco-nutrient agar slants growth was good with entire edges, smooth and deep yellow. Growth occurred in Difco nutrient broth, Ayers, Rupp & Johnson medium containing sucrose, lactose broth, Koser citrate medium, Clara's medium, formate ricinoleate broth, and lauryl tryptose broth. A faint turbid growth occurred in Fermi's solution in 2 weeks. A soft curd formed in skim milk which became peptonized in 4 weeks, leaving a clear, reddish whey; the reaction was alkaline. No growth occurred on Endo, bismuth sulfide or desoxycholate agars. Krumwiede triple sugar agar supported pale orange, mucoid growth and the medium turned red.

Physiological Reactions: Gelatin was liquefied in 1 week, and a solidified polypectate medium showed liquefaction in the area along the streak. A positive test for H_2S occurred in tryptone and thiotone broths. In Difco nitrate broth medium the pathogen gave a negative test for nitrite in 48 hours. In deep cultures of nutrient agar, plus dextrose, the pathogen grew as a strict aerobe. All cultures were catalase-positive and urease-negative. The MR-VP tests were both negative as also was the test for indole production. Starch was hydrolyzed. On Loeffer's blood serum the Florida isolates produced a dark brown pigment and liquefied the medium in contrast to the cultures of X. poinsettiaecola from ATCC and England which produced little or no pigmentation and no liquefaction.

The poinsettia bacterium utilized several carbon sources producing acid from the following: arabinose, xylose, glucose, fructose, galactose, mannose, lactose, sucrose, maltose, melibiose, raffinose, dextrin, mannitol and cellobiose. Inositol, melezitose, ethyl alcohol and inulin were not utilized. Feeble acid production occurred in salicin and trehalose. An alkaline reaction occurred in a basal medium containing sodium citrate and sodium malonate; sodium tartrate was not utilized. Growth occurred in Difco nutrient broth supplemented with 3% NaCl, but not with 5%. The thermal death rate was about 54° C.

Cross-inoculation Tests: Typical water-soaked, brown lesions developed on leaves of 'Barbara Ecke Supreme' and 'Ecke's Flaming Sphere' poinsettias when artificially inoculated with cultures of X. poinsettiaecola. The leaves were lightly dusted with carborundum before inoculation. In 1960 the Florida isolates from poinsettia were about equal in virulence to the cultures of X. poinsettiaecola obtained from ATCC and England. In 1961 the Florida isolates were consistently less pathogenic to poinsettia leaves, producing fewer and smaller lesions than those produced by X. poinsettiaecola under identical conditions. In the spring of 1962 all the Florida isolates were either weakly virulent or avirulent, while the cultures of X. poinsettiaecola from England and ATCC still proved highly virulent to poinsettia. The reason for this change in virulence is not clearly understood since all cultures were maintained on identical media and incubated or stored under identical conditions. Attempts are being made to increase the virulence of the Florida isolates by repeated inoculations and isolations and re-inoculation on susceptible poinsettia varieties.

Cultures of Xanthomonas obtained from R. S. Dickey, Cornell University, including X. pelargonii (Brown) Starr & Burkholder (XP39), X. begoniae (Takimoto) Dowson (XB8), X. malvacearum (E. F. Smith) Dowson (XM14), and X. campestris (Pammel) Dowson (XC16) were all found nonpathogenic to leaves of poinsettia. Likewise, X. vesicatoria (Doidge) Dowson from tomato leaves, and X. dieffenbachiae (McCulloch & Pirone) Dowson (XD101 from M. P. Starr, University of California) failed to produce lesions on poinsettia leaves. No attempts were made to adapt these pathogens to poinsettia using the method described by Dye (2).

Taxonomy of the Pathogen: The pathogen causing leaf spot of poinsettia in Florida clearly belongs in the genus Xanthomonas since it is a non-spore forming, gram-negative, short rod having a single polar flagellum, and produces abundant, slimy, yellowish growth on freshly prepared potato-dextrose agar. In all the morphological, cultural and physiological tests performed, with the exception of Loeffer's blood serum medium, the Florida isolates from poinsettia compared favorably with X. poinsettiaecola. In cross-infection studies X. poinsettiaecola produced similar lesions on poinsettia leaves to the Florida isolates and indistinguishable from natural infections. The Florida isolates, however, became weakly virulent or avirulent within 2 years, whereas the cultures of X. poinsettiaecola from ATCC and England still remained highly virulent even though in culture for a longer time. Even so, the bacterium isolated from leaf spot lesions of poinsettia in Florida is now considered to be a strain of X. poinsettiaecola. This is believed to be the first report of the disease in the United States.

CONTROL

Five plants of 'Ecke's Flaming Sphere' and four plants of 'Henriette Ecke Double' were planted in each of 20 plots in outdoor beds provided with overhead irrigation. In replicated treatments, sprays were applied to the above-named plants at weekly intervals over a period of 11 months. Since natural leaf spot infections were noted in the early spring of 1961 no attempts were made to artificially inoculate the plants. The final data obtained on January 19, 1962 showed that no disease occurred in plots sprayed with Tribasic Copper Sulfate (53% copper as metallic) at 4 pounds/100 gallons, a trace occurred in plots sprayed with either Agri-mycin 500 (10% streptomycin sulfate, 1% oxytetracycline, + basic copper sulfate) at 4 pounds/100 gallons, or Agri-mycin 100 (15% streptomycin sulfate, 1.5% oxytetracycline) at 60 ppm streptomycin. A moderate amount of disease occurred in plots sprayed with Agri-Strep (37% streptomycin sulfate) at 60 ppm. All materials except Agri-Strep were significantly better than the unsprayed controls. When control measures are warranted, efforts should be made to avoid keeping the foliage wet for long periods. A weekly spray of Tribasic Copper Sulfate at 4 pounds/100 gallons or its equivalent should provide adequate control of the disease.

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ISOLATION AND IDENTIFICATION OF TOMATO RING SPOT VIRUS ASSOCIATED WITH ROSE PLANTS AND ROSE MOSAIC VIRUS[1]

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Abstract

Tomato ring spot virus, Fulton's culture of rose mosaic virus, and three Oregon isolates from rose mosaic plants all showed similar symptoms on *Chenopodium*, black cowpea, French bean, and cucumber. Antiserum prepared from tomato ring spot virus reacted with Fulton's rose mosaic virus, and antiserum prepared from Fulton's rose mosaic virus isolate reacted with tomato ring spot virus and the three Oregon rose mosaic virus isolates. Apparently the disease known as rose mosaic is caused by strains of tomato ring spot virus.

INTRODUCTION

New varieties of rose plants, introduced by nurseries from various parts of the United States, already are showing evidence of virus patterns. The same virus patterns can be noted in many of the older varieties, and those not showing virus symptoms could be carrying viruses. This observation led to a study of the rose virus problem supported by funds from the Research Council of the Oregon Nurserymen's Association.

Fulton (4) isolated and studied rose mosaic virus (RMV) on herbaceous hosts. A culture of this virus was used as a type culture in these studies. By using Fulton's techniques, and by developing others, we readily obtained similar mechanically transmissible cultures of a virus from rose plants showing RMV symptoms. Both Fulton's isolate and the Oregon isolates produced symptoms on diagnostic hosts suggestive of tomato ring spot virus. AMTC 13, of tomato ring spot virus (TomRSV) maintained at Oregon State University, and the cherry isolates of the Eola strains of the virus (9), which were reported to be strains of TomRSV, were available for comparisons.

ISOLATION FROM ROSES

Various attempts were made to isolate a virus from rose leaves and petals by mechanical inoculations. Natural virus inhibitors apparently inactivate the virus during maceration and extraction. Alfalfa mosaic virus was added to rose tissue extracts before inoculating cowpeas to test for virus inactivation. The young terminal rose leaves reduced the infectivity more than the mature leaves. Various chemicals such as EDTA, cysteine HCl, ascorbic acid, sodium sulfite, KCN, gelatin solution and nicotine sulfate were used in an attempt to prevent this inactivation of RMV when isolating from rose leaves, but none of the treatments was successful. When Lovell peach seedlings or *Vinca rosea* were inoculated with chip- or T-buds from infected rose plants, a virus was readily isolated from the young tip leaves of these hosts 30 to 45 days later. Black cowpea (*Vigna sinensis*), *Cucurbita pepo* var. White Scallop, and cucumber (*Cucumis sativus* var. Chicago Pickling) were used to isolate the viruses. When cucumber or squash were included as host plants, two distinct viruses were often isolated. One of these proved to be the Prunus ring spot virus (PRSV). A survey of 55 hybrid tea roses showing rose mosaic symptoms produced 25 positive Shiro-fugen tests for PRSV. The RMV was readily separated from this virus by using black cowpea or French bean as the host.

Eight different cultures of RMV were isolated, but after preliminary studies, three were selected that showed the greatest difference in host reactions. These three Oregon RMV isolates, Fulton's isolate and tomato ring spot virus were the viruses studied and compared. The Oregon isolates were designated as P-4, V-1B, P-2, Fulton's isolate as F-1 and tomato ring spot AMTC 13 as TomRSV for convenience in the following experiments.

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[1] Technical Paper No. 1576, Oregon Agricultural Experiment Station. Taken in part from Ph. D. thesis data of senior author.

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COMPARISON ON HERBACEOUS HOSTS

Chenopodium amaranticolor: All isolates caused local lesions on the inoculated leaves of C. amaranticolor and later systemic symptoms developed. The age of the plant or individual leaf was more influential in determining symptom variation than were the different isolates. Small necrotic flecks, rings or chlorotic spots were usually produced as local lesions. Systemic symptoms consisted of chlorotic lines or ring patterns which were frequently associated with the veins.

Black cowpea (Vigna sinensis): Black cowpea was readily infected with all cultures of the virus. The inoculated primary leaves showed only faint chlorotic lesions which often were indistinct. When inoculum of high virus titer was used, systemic symptoms appeared on the first trifoliate leaf. Fulton's isolate produced brilliant golden patterns in the form of rings, golden veins, or veinbanding patterns. The other strains produced mild chlorotic patterns and some necrosis. A high virus titer could be obtained from the young leaves.

French bean (Phaseolus vulgaris): All isolates were compared in the French bean varieties Bountiful and Sutter's Pink. Distinct chlorotic lesions developed in the inoculated primary leaves. The systemically infected trifoliate leaves were smaller, distorted, and mottled with scattered necrotic spots or flecks.

Cucumber (Cucumis sativus): All isolates produced chlorotic spots on the inoculated cotyledons of cucumbers. Necrotic and chlorotic patterns developed on the first true leaf, the growing points became necrotic, and the plants generally died within 1 1/2 to 2 weeks following inoculation.

SEROLOGICAL COMPARISON OF RMV AND TomRSV

The similarity of RMV and TomRSV and their effect on common host plants suggested a close relationship between the two viruses. Antiserum was prepared from Fulton's isolate of RMV and from AMTC of TomRSV. Cross-precipitin and micro-precipitin tests established the serological relationship of these two viruses. Clarified juice extracts of the three Oregon RMV isolates also reacted in cross-microprecipitin tests with antiserum of Fulton's RMV. Precipitation occurred in the normal manner with all Oregon isolates, while the controls of normal serum and of antigen prepared from healthy plants failed to produce any reaction. Fulton's isolate of RMV shares many of the same antigens as TomRSV and the Oregon isolates of RMV. The TomRSV antiserum also was precipitated when reacted with the Eola cherry isolate of TomRSV.

DISCUSSION

These tests and observations show that RMV and TomRSV are closely related and justify further consideration. Apparently many rose stocks are infected with this virus. Rose plants are distributed throughout the world, and RMV has been reported from all the major continents (2, 3, 5, 7, 8). The TomRSV has been reported to be soil-retained and nematode-transmitted (1), and from some infected host's seed, transmission was high (6). The wide host range of the virus includes peaches, cherries, and a number of annual plants. Many other perennial plants are probably infected.

Methods of eradicating the virus from infected plants will have to be developed. The virus should be included in any nursery improvement or quarantine program.

The effects of the virus on rose and other hosts need to be studied. On some rose varieties virus symptoms are expressed on at least one or more leaves, and even when the virus is in a latent or symptomless condition, the vigor, longevity, hardiness, flower color, and other production aspects of roses are probably affected.

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RESISTANCE OF ALFALFA TO TWO SPECIES OF ROOT-KNOT NEMATODES[1]

John H. O'Bannon and Harold W. Reynolds[2]

Summary

Seedlings of one breeding line of Sirsa alfalfa and 13 breeding lines of African were tested for resistance to the Javanese root-knot nematode, Meloidogyne javanica javanica, and the cotton root-knot nematode, M. incognita acrita. The 13 selections from African were highly resistant to both species as compared with the Lahontan check, and the one Sirsa selection had intermediate resistance.

African alfalfa (Medicago sativa) has been shown to be very resistant to the two most common species of root-knot nematodes in Arizona, the Javanese (Meloidogyne javanica javanica (Treub) Chitwood) and the cotton root-knot nematode (M. incognita acrita Chitwood) (4). No information on root-knot resistance of the Sirsa selection is available. This paper reports tests on 13 breeding lines of African alfalfa and one selection of Sirsa selected for resistance to the spotted alfalfa aphid and high forage production[3] for the purpose of determining their reactions in the seedling stage to these two species of root-knot nematodes.

MATERIALS AND METHODS

One hundred twenty-eight 4-inch pots containing soil free from root-knot nematodes were embedded in sand in the greenhouse bench. To provide a relatively uniform and almost optimum temperature for development of the root-knot nematode, a mean temperature of 27° C was maintained by an electric heating cable embedded in the sand beneath the pots. Sixty-four pots were inoculated with the Javanese root-knot nematode and 64 with the cotton root-knot nematode. The inoculum, which consisted of viable larvae in water, was secured in accordance with the procedure outlined by Godfrey (1). Approximately 2100 M. javanica javanica larvae and 2300 M. incognita acrita larvae were added to the soil of each pot at the time of planting. The 64 pots in each group were spaced sufficiently far from the other group to prevent contamination through watering or larval migration.

In each group, 64 pots (4 seeded with each of the 13 breeding lines of African, one of Sirsa alfalfa, and two check varieties) were randomized in four blocks of 16 pots each. The checks were African and Lahontan alfalfa. Approximately seven plants were grown in each pot for 75 days. The plants were harvested and washed lightly to remove excess soil. Each plant was given a root-knot rating (5) based on the extent of galling and averages were calculated by the McKinney formula (3) as modified by Horsfall and Heuberger (2). The rating for each plant was determined with the aid of a dissecting microscope.

RESULTS

All entries of alfalfa were infected to some degree by each of the two species of root-knot nematodes (Table 1). The 13 selections of African and the African check were all rated less than 15 for both species, indicating that the selections have the high degree of resistance to the Javanese and cotton root-knot nematodes characteristic of the parent strain. Lahontan, the variety grown extensively in areas where the alfalfa stem nematode is a problem, was moderately susceptible to both root-knot nematode species. Entry M-56-11, a Sirsa selection which has spotted alfalfa aphid resistance, proved to be intermediate in susceptibility.

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Arizona Agricultural Experiment Station.

[2] Nematologists, Agricultural Research Service, Cotton Research Center, Tempe, Arizona.

[3] Seed was furnished by Dr. M. H. Schonhorst, University of Arizona, Tucson, and Dr. M. W. Nielson, Entomology Research Division, Agricultural Research Service, United States Department of Agriculture, Mesa, Arizona. The breeding lines were developed cooperatively by State and Federal alfalfa workers in Arizona, California and Nevada.

Table 1. Average resistance ratings of 14 breeding lines of alfalfa and 2 check varieties to 2 species of root-knot nematodes.

Entry No. and variety	Root-knot index[a]: Javanese root-knot nematode	Root-knot index[a]: Cotton root-knot nematode
1. African (check)	3.5a[b]	1.9a
African selections:		
2. E-22	6.3a	2.0a
3. M-5-9	3.9a	2.9a
4. M-5-44	7.3a	3.3a
5. E-23	6.4a	4.6a
6. Y-5-7	2.8a	5.3a
7. P-8	6.6a	5.3a
8. P-14	14.4a	5.5a
9. B-40	7.7a	5.8a
10. P-2	6.1a	6.1a
11. N-2038	4.3a	6.3a
12. AP-12	6.1a	6.7a
13. B-41	3.4a	9.1a
14. Y-5-41	10.7a	10.5a
Sirsa selection:		
15. M-56-11	30.0b	27.9b
16. Lahontan (check)	67.1c	51.8c

[a] Root-knot index: 0 = no infection; 1-25 = trace to light infection; 26-50 = light to moderate infection; 51-75 = moderate to heavy infection; 76-100 = heavy to severe infection.

[b] Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 1% level.

DISCUSSION

Since alfalfa is grown regularly in rotation with root-knot nematode susceptible crops, a high nematode population can build up and be maintained by a susceptible variety of alfalfa. With this reservoir of nematodes in the soil, susceptible crops such as cotton and melons will be damaged. Stands of susceptible alfalfa varieties are also weakened as infection of the primary and secondary roots can and does occur in the seedling stage. Willhite and Smith (6) observed that heavy root-knot infections influence the type of root system and the quantity and quality of forage produced.

With highly resistant varieties, seedling damage is diminished and a vigorous root system develops. The nematode population is reduced, enabling succeeding crops following in the rotation to remain relatively free from root-knot nematode damage, depending upon the degree of susceptibility.

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THE COMPARATIVE EFFECTS OF VARIOUS FUNGICIDE PROGRAMS ON FRUIT NUMBERS AND YIELDS OF APPLE TREES

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Summary

Phenylmercury acetate and some other commonly used fruit fungicides, when applied in a complete early season schedule from the delayed dormant through the first cover spray period, caused highly significant reductions in fruit numbers and in yields of 5- to 11-year-old Cortland apple trees. Phenylmercury acetate, the phenyl mercury most used in the tests, when applied as many as four times, but only in the prebloom period, did not reduce fruit numbers or lower apple yields. A single phenylmercury acetate spray applied at first cover, or two sprays applied, one at petal fall and one at first cover, caused a reduction in fruit numbers almost as great as the complete program of this material. In contrast, two methyl mercury fungicides, methylmercury dicyandiamide (Panogen Apple Spray) and methylmercury 8-hydroxyquinolinate (Ortho LM Apple Spray) caused neither a reduction in fruit numbers nor reduced apple yields even when applied in the complete schedule through first cover. A complete early season schedule of one-half strength phenylmercury acetate plus one-half strength glyodin caused defruiting and lowered yields of apple trees as great as the same schedule of full strength phenylmercury acetate alone.

INTRODUCTION

Organic mercury compounds and certain other eradicant-type fungicides have provided apple growers with effective tools for the emergency control of apple scab where, for one reason or another, the application of conventional protective fungicides was not adequately timed. Since the eradicant materials can be effectively applied within a reasonable time after rains and thus after infection, their use in this way frequently enables the apple grower to avoid serious damage which would otherwise result from spray timing errors. The organic mercury compounds, mostly of the phenyl type, are widely used as emergency sprays, and have even been employed to the exclusion of other fungicides during the early scab season in what has been termed an "after rains" or "after infection" spray schedule. Soon after the organic mercuries came into common use, reports were heard that they caused failure in "fruit set" and that apple yields were correspondingly lowered. This damage apparently occurred even in the absence of visible leaf injury which occasionally was observed when either an overdose of fungicide was applied or when the material was used later in the season than recommended. To the writer's knowledge, the reports were based only on grower observations. There was no good experimental evidence to substantiate or disprove them. The reports grew in number and persisted, however, and in 1955 the author initiated a series of experiments to determine the effects of early season organic mercury and other fungicide sprays upon fruit numbers and yields of apple trees.

PROCEDURE

The apple trees used for the major part of the work were of the Cortland variety and were planted at Wooster, Ohio in 1950. A randomized block design was used, with single tree plots replicated from 6 to 16 times, depending each year upon the number of treatments and the number of trees available. The same treatments were not applied to the same trees year after year, but the trees were re-randomized each spring. The effects of fungicides upon fruit numbers and apple yields which were measured by these experiments, therefore, are those for the current season only. The tests were not designed to measure carry-over effects of previous years' treatments.

Fungicides were applied each year, starting at either the delayed dormant or prepink stage of bud development. The organic mercury compounds were applied in an "after rains" schedule. All other fungicides were applied in a "protective" schedule, as nearly as possible just prior to predicted rains. The number of sprays applied from the start of the season through the first cover period varied somewhat from year to year depending on weather conditions. The number of sprays applied in the "after rains" schedule differed also, in some seasons, from the number applied in the "protective" schedule. Five applications of each mercury spray were made in the complete schedules from the delayed dormant through first cover period in 1955, seven in 1956, five in 1957, four in 1958, five in 1959, five in 1960, and six in 1961. Seven sprays of the nonmercury materials were applied during the same period in 1955, six in 1956, six in 1957, five in 1958, six in 1959, five in 1960, and six in 1961. All sprays were thoroughly applied at 500 pounds' pressure with a conventional hydraulic sprayer, equipped with hand operated guns. Insecticides were added to all treatments alike as needed for insect control. Dieldrin (50% WP) 1/2 pound per 100 gallons was used in the petal fall and first cover sprays in 1960 for curculio control. Lead arsenate 3 pounds per 100 gallons was used in petal fall and first cover in all other years. DDT (50% WP) 2 pounds per 100 gallons was added to all sprays after first cover for codling moth control. Early season control of mites and aphids was obtained through the application of annual dormant sprays of superior oil plus dinitro.

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To determine precisely the effects of the various fungicide programs on fruit numbers, four limbs of average vigor on each tree were tagged each year while the trees were in bloom. Preferably, limbs were selected which bore between 100 and 200 blossom clusters. The number of blossom clusters on each limb was recorded. Later, after all "June fruit drop" had occurred, the number of fruits remaining on each tagged limb was recorded. Fruit counts were usually made during the last week of June. Precise data were thus obtained, expressing for each test tree the number of fruits retained per 100 blossom clusters present during the bloom period. Yield data were obtained each year at harvest. Since unsprayed trees could not be used as controls for these experiments, owing to loss of fruit through scab infection, a captan treatment was included each year as a standard for comparison; captan was selected because of its wide use in commercial orchards and because it appeared to cause little defruiting.

RESULTS AND DISCUSSION

The results of all 7 years' experiments are shown in Tables 1, 2, 3, and 4.

Table 1 shows that certain complete early season fungicide programs definitely caused reductions in numbers of fruits retained by 5- to 11-year-old Cortland apple trees. Table 3 further shows that this defruiting was directly reflected in lowered apple yields. Phenylmercury acetate, at standard dosages and when applied in a complete early season schedule from delayed dormant through the first cover period, caused highly significant defruiting in 5 years of 7. Highly significant or significantly lowered yields occurred in 6 of 7 years. Certain other fungicide programs also caused defruiting and lowered yields of young Cortland apple trees. Dichlone, in the seasons tested, caused defruiting and lowered apple yields as extreme as those caused by the phenylmercury fungicides. The combination of phenyl mercury and glyodin at one-half strength each, a combination widely used by growers in an attempt to avoid the phytotoxicity attributed to the use of full strength mercuries, caused fully as much defruiting and lowered yield as did the full strength phenyl mercury alone.

A combination of sulfur and dichlone (Phybam S) caused highly significant defruiting in 1957 and 1958, the only years it was tested. Glyodin reduced fruit numbers on 9-year-old Cortland in 1959, in 1 year of 5. Lowered yields, in relation to those resulting from the captan schedule, occurred on the glyodin-sprayed trees in 4 of 5 years. Microfine dry wettable sulfur caused lowered yields in 2 of 4 years. Complete early season schedules of dodine, at dosages of more than 1/2 pound per 100 gallons, caused highly significant defruiting and lowered yields of Cortland apple trees in the single year each dosage was tested. Dodine, at dosages of 1/2 pound or less, did not cause defruiting or reduced yields.

Of the fungicides compared with captan in these tests, only two at no time caused defruiting or lowered yields of young Cortland trees. Two methyl mercury compounds, methylmercury dicyandiamide (Panogen Apple Spray) and methylmercury 8-hydroxyquinolinate (Ortho LM Apple Spray) caused no defruiting or lowered yields in the several years each was tested. In fact, in 1 year (1960) the application of a full early season schedule of methylmercury 8-hydroxyquinolinate resulted in an apple yield highly significantly greater than that of trees sprayed throughout with captan.

As shown by Table 2, prebloom only schedules of phenylmercury acetate (three to four applications) did not cause significant defruiting of young Cortland apple trees. Only in one of three seasons did the prebloom application of this material result in a reduction in yield (Table 4). It was only when the sprays of phenylmercury acetate were continued through petal fall and first cover that fruit numbers and yields were reduced. Single applications of phenylmercury acetate in bloom or petal fall did not lower fruit numbers or yields. A single spray of phenyl-

Table 1. Effects of fungicides on numbers of fruits retained by 5- to 11-year-old Cortland apple trees.

Fungicide in 100 gallons through first cover	Number of fruits retained per 100 blossom clusters 1955	1956	1957	1958	1959	1960	1961
Captan 2 lb (standard for comparison)	56.4	6.4	20.6	26.7	26.6	26.3	51.9
Phenylmercury acetate (Phix 4 oz 1955, 1956, 1957, 1958; 3 oz 1959, 1960, 1961)	24.4**	0.6	6.4**	12.2**	5.4**	16.1	25.6**
Phenylmercury acetate (Phix) 2 oz (1 1/2 oz 1961) plus glyodin 1 pt	----	---	----	8.9**	----	20.7	25.4**
Phenylmercury monoethanol ammonium acetate (Puratized Apple Spray) 1/2 pt	----	0.9	----	----	----	----	----
Phenylmercury triethanol ammonium lactate 7 1/2% (Puratized Agricultural Spray) 3/4 pt	----	4.4	----	----	----	----	----
Methylmercury dicyandiamide (Panogen Apple Spray) 1 pt	----	19.9**	41.0**	27.5	----	----	----
Methylmercury 8-hydroxyquinolinate (Ortho LM Apple Spray) 1/2 pt	----	---	----	33.9	28.1	41.5	69.2
Glyodin 2 pt	----	4.5	17.7	25.9	12.7**	21.4	----
Microfine Sulfur 6 lb	29.6**	18.9**	19.7	18.6	----	----	----
Dichlone (Phygon XL) 1/4 lb (1/2 lb 1957)	----	----	2.3**	12.7**	9.5**	----	----
Dichlone -- Sulfur (Phybam S) 4 lb	----	----	7.6**	7.7**	----	----	----
Thiram (Thylate) 2 lb	----	----	10.4*	----	----	----	----
Dodine (Cyprex dodine 65 W) 1 1/2 lb	----	----	6.2**	----	----	----	----
Dodine (Cyprex dodine 65 W) 3/4 lb	----	----	----	13.8**	----	----	----
Dodine (Cyprex dodine 65 W) 5/8 lb	----	----	----	----	12.1**	----	----
Dodine (Cyprex dodine 65 W) 1/2 lb	----	----	----	----	----	32.8	----
Dodine (Cyprex dodine 65 W) 3/8 lb	----	----	----	----	----	----	38.1
LSD .05	9.2	9.1	9.6	8.3	8.6	NS	19.1
LSD .01	12.4	12.2	12.8	11.0	11.5	NS	25.5

*Differs from captan standard at 5% level.
**Differs from captan standard at 1% level.

Table 3. Effect of fungicides on yields of 5- to 11-year-old Cortland apple trees.

Fungicide in 100 gallons	Pounds of apples per tree 1955	1956	1957	1958	1959	1960	1961
Captan 2 lb through first cover, then 1 lb	122.9	47.9	167.0	222.2	394.9	365.8	460.5
Phenylmercury acetate (Phix) 4 oz (3 oz 1959, 1960, 1961) through first cover, then captan 1 lb	65.2*	7.1**	47.4**	180.0	158.6**	193.5**	328.5*
Phenylmercury acetate (Phix) 2 oz (1 1/2 oz 1961) plus glyodin 1 pt through first cover, then glyodin 1 pt	-----	-----	-----	135.0*	-----	259.5**	338.2*
Methylmercury 8-hydroxyquinolinate (Ortho LM Apple Spray) 1/2 pt through first cover, then captan 1 lb	-----	-----	-----	272.8	393.6	461.2**	541.5
Methylmercury 8-hydroxyquinolinate (Ortho LM Apple Spray) 1/4 pt plus captan 1 lb through first cover, then captan 1 lb	-----	-----	-----	-----	-----	-----	521.2
Glyodin 2 pt through first cover, then 1 pt	-----	25.8*	132.1*	253.1	248.6**	245.5**	-----
Microfine sulfur 6 lb through first cover, then 5 lb	63.4**	36.7	130.9*	202.5	-----	-----	-----
Dichlone (Phygon XL) 1/4 lb through first cover, then captan 1 lb	-----	-----	-----	126.5**	189.5**	-----	-----
Dodine 3/4 lb through first cover, then 1/2 lb	-----	-----	-----	128.6**	-----	-----	-----
Dodine 5/8 lb through first cover, then 3/8 lb	-----	-----	-----	-----	174.4*	-----	-----
Dodine 1/2 lb through first cover, then 1/4 lb	-----	-----	-----	-----	-----	348.0	-----
Dodine 3/8 lb throughout season	-----	-----	-----	-----	-----	-----	469.5
Dodine 1/4 lb throughout season	-----	-----	-----	-----	-----	-----	479.2
LSD .05	18.6	11.7	32.7	67.8	88.2	63.4	117.4
LSD .01	25.1	15.6	44.0	89.7	117.7	84.9	156.8

*Differs at 5% level from captan standard.
**Differs at 1% level from captan standard.

Table 2. Effect of number of phenylmercury acetate sprays and their time of application on number of fruits retained by 6- to 11-year-old Cortland apple trees.

Fungicide and timing of sprays	Numbers of fruits retained per 100 blossom clusters 1956	1957	1958	1960	1961
Captan 2 lb through first cover, then 1 lb	6.4	20.6	26.7	23.6	51.9
PMA[a] through prebloom	8.3	27.9	29.6	24.2	35.8
PMA through bloom	2.8	16.4	22.8	----	----
PMA through petal fall	0.4	10.2*	12.4**	----	----
PMA through first cover	0.6	6.4**	12.2**	16.1	25.6**
PMA in bloom only (1 application)	5.1	29.2	25.8	----	----
PMA in petal fall only (1 application)	---	----	23.5	----	----
PMA in first cover only (1 application)	---	----	12.8**	----	----
PMA in bloom and petal fall (2 applications)	1.1	11.8	----	----	----
PMA in petal fall and first cover (2 applications)	---	9.0*	----	----	----
PMA in bloom, petal fall, and first cover (3 applications)	0.0	----	----	----	----
LSD .05	NS	9.6	8.3	NS	19.1
LSD .01	NS	12.8	11.0	NS	25.5

[a] Phenylmercury acetate 22% (Phix) 4 oz per 100 gallons (3 oz 1960, 1961). Captan 2 lb per 100 gallons used in all other sprays from delayed dormant through first cover, then captan 1 lb.
*Differs at 5% level from captan standard.
**Differs at 1% level from captan standard.

Table 4. Effect of number of phenylmercury acetate sprays and their time of application on yields of 8-, 10-, and 11-year-old Cortland apple trees.

Fungicide and timing of sprays	Pounds of apples per tree 1958	1960	1961
Captan 2 lb through first cover, then 1 lb	222.2	365.8	460.5
PMA[a] through prebloom	272.8	292.7*	506.2
PMA through bloom	275.6	-----	-----
PMA through petal fall	196.8	-----	-----
PMA through first cover	180.0	158.6**	193.5**
PMA in bloom only (1 application)	289.7	-----	-----
PMA in petal fall only (1 application)	230.6	-----	-----
PMA in first cover only (1 application)	192.9	-----	-----
LSD .05	NS	63.5	117.4
LSD .01	NS	84.9	156.8

[a] Phenylmercury acetate 22% (Phix) 4 oz (3 oz 1960, 1961) per 100 gallons. Captan 2 lb per 100 gallons in remainder of sprays through first cover period. Captan 1 lb per 100 gallons in all sprays after first cover.
*Differs at 5% level from captan standard.
**Differs at 1% level from captan standard.

mercury acetate in first cover resulted in highly significant defruiting in 1958. From the results obtained in these tests, it would appear that complete prebloom schedules of phenylmercury acetate can be used on Cortland apples without danger of crop loss, and that single phenylmercury spray applications could be made as late as petal fall without serious danger of crop loss.

Most of the author's work has been done on trees between the ages of 5 and 11 years. Some experiments were performed on mature apple trees (20 or more years old). The limited nature of this work prompts the author to state only that the defruiting effects of fungicides on mature trees appears to be less pronounced than on young trees. Furthermore, the moderate reductions in fruit numbers resulting from the use of phenylmercury acetate on mature trees appeared to have little effect on yield. Undoubtedly, there are varietal differences in reaction to the application of certain fungicides. The use of such materials as the phenyl mercuries and dichlone after bloom on Red Delicious of any age would be open to question in many situations since this variety frequently "sets" sparse crops of fruit.

The work reported here has dealt entirely with the quantitative effects of various fungicide programs on fruit numbers and yield of apple trees. No attempts were made to determine the mechanism or mechanisms by which defruiting was brought about. The results indicate, however, that the defruiting caused in these tests was not due to influence of the fungicides on pollination. Sprays applied after apple bloom caused more defruiting than those applied during the pollination period. Also, the fruit loss attributable to fungicide effect occurred mostly after the fruits were 1/2 inch or more in diameter. Scab control with all fungicides was excellent throughout the tests, therefore, scab infection played no appreciable part in the defruiting which occurred in the experiments reported here.

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TOBACCO-ETCH VIRUS ON PEPPERS AND TOMATOES IN THE LOWER RIO GRANDE VALLEY OF TEXAS

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Summary

Tobacco-etch virus is reported on peppers and tomatoes in the Lower Rio Grande Valley of Texas. Symptoms produced on selected host species are described. The green peach aphid was an efficient experimental vector.

INTRODUCTION

Bell-peppers (Capsicum annuum) produced in the Lower Rio Grande Valley of Texas mostly are of the 'Yolo Wonder' types. Resistance to tobacco mosaic virus (TMV) was demonstrated in this variety (9). Another mottle virus was common in 'Yolo Wonder' and often limited production by its effects on fruit. The virus appeared also on tomatoes (Lycopersicon esculentum). Indexing to specific indicator plants through 1959-60-61 from numerous field-infected plants in spring and fall plantings showed this virus to be a strain of tobacco-etch virus (TEV). During this period it was the most common and widespread virus on peppers and tomatoes in the Lower Rio Grande Valley.

The green peach aphid (Myzus persicae (Sulz.)) was an efficient experimental vector.

SYMPTOMATOLOGY

Symptoms resembled those incited by potato Y virus (PYV) (10, 11) or by TMV in 'California Wonder' pepper (1, 5, 8). On 'Yolo Wonder' plants early symptoms consisted of veinclearing; dark-green veinbanding, and eventually a diffuse, mild mottle on reduced size of terminal leaves followed (Fig. 1, A). Infected fruits were roughened, distorted, and had chlorotic spots or streaks. Early-set fruits, particularly in fall-grown peppers, sometimes showed brown to black, flattened or sunken blotches with indefinite margins. Frequently the discoloration extended into the fleshy fruit wall. Symptoms resembled those described as internal browning or graywall on tomato fruit (3). Affected fruits were not always on virus-infected plants, but TEV was sometimes isolated. It is not certain whether these fruit symptoms are part of the TEV symptom syndrome or a response to unfavorable environmental conditions.

On tomato, initial symptoms were mild veinclearing in young leaves; mild mottle with pronounced epinasty followed (Fig. 1, B). Symptoms were not apparent on tomato fruit.

MATERIALS AND METHODS

Terminal growth was removed from field-infected plants and triturated in a mortar. Crude extract was diluted approximately 1/10 with 0.1M K_2HPO_4 neutral buffer. Different virus isolates were maintained in Nicotiana tabacum 'Kentucky Burley 16.' Mechanical inoculations were made in a glasshouse with the aid of new 6-inch wooden pot labels or with sterile cotton-covered toothpicks dipped into undiluted, freshly expressed sap from infected tobacco plants and stroked lightly over leaves of test plants previously dusted with carborundum (400).

Green peach aphids were maintained on healthy 'Yolo Wonder' pepper plants. Aphids were caged (4) on infected 'Yolo Wonder' plants for 3 hours and then transferred to healthy plants and permitted a 1-hour test feeding.

RESULTS AND CONCLUSIONS

Different workers have suggested indicator plants for differentiating viruses commonly affecting peppers (1, 2, 5). TEV causes wilting in 'Tabasco' pepper (Capsicum frutescens) (1, 5), severe mottle with blistering and distortion in Jimson weed (Datura stramonium) (1, 5, 7, 8), mild chlorotic mottle and etch patterns in Nicotiana tabacum and N. glutinosa (1,2,5,8). These reactions differentiate TEV from TMV, PYV, and cucumber mosaic virus (CMV) in these hosts.

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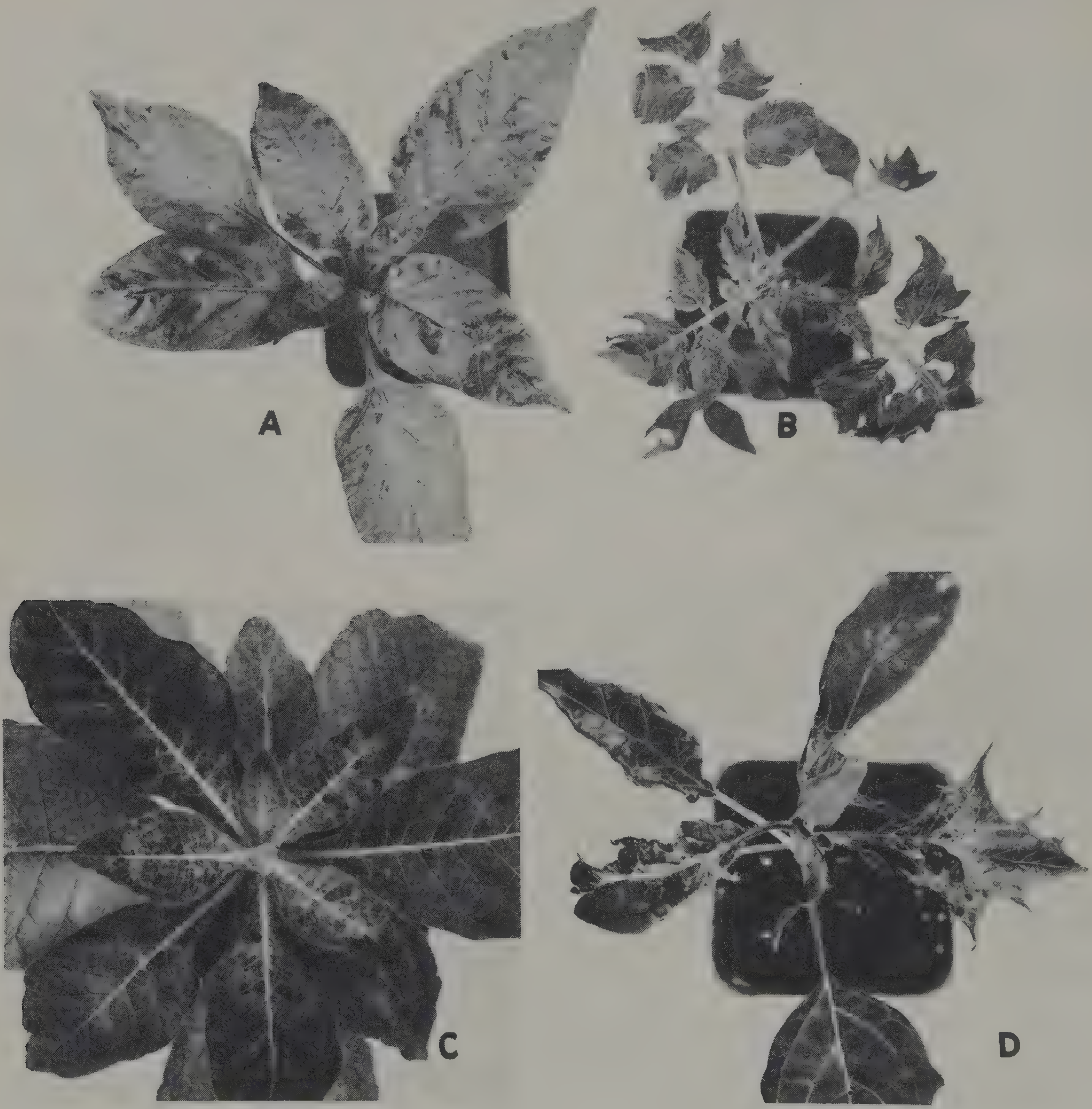

FIGURE 1. Symptoms caused by tobacco-etch virus. A -- Pepper 'Yolo Wonder.' B -- Tomato 'Rutgers.' C -- Turkish tobacco. D -- Datura stramonium.

Species inoculated and their reactions to TEV isolates from pepper and tomato are listed in Table 1.

All virus isolates produced similar reactions in Turkish tobacco. Initial symptoms were veinclearing on inoculated leaves; veinbanding and diffuse chlorotic mottle followed. Young leaves eventually showed chlorotic spots, which became brownish, partially circular to segmented, with etch designs. Symptoms faded in older leaves, which became chlorotic and very mildly mottled (Fig. 1, C). Etched patterns were not distinct in N. glutinosa, but principal symptoms were veinclearing, wavy leaf margins, and systemic rugosity on some inoculated plants. Jimson weed always developed severe mottle and leaf distortion in response to each TEV isolate (Fig. 1, D). Very young 'Tabasco' plants sometimes showed indistinct veinclearing before wilting. Older inoculated plants wilted as rapidly as plants inoculated in the primary-leaf stage. Wilted plants did not recover and ultimately died.

Holmes (6) used Physalis peruviana as a local-lesion indicator species for quantitative measurement of a severe strain of TEV, but indicated that a mild strain produced only mottling on this species. In the present studies, TEV isolates produced veinclearing, epinasty,

Table 1. Symptoms produced on selected species by isolates of TEV from pepper and tomato in the Lower Rio Grande Valley.

Test species	Symptoms
Capsicum annuum	
'California Wonder' (1, 5, 7, 8)[a]	veinclearing, veinbanding, severe mottle, leaf crinkling, distortion
'Yolo Wonder'	do.
C. frutescens	
'Tabasco' (1, 5)	severe wilting, sometimes early mild veinclearing, root necrosis
Cassia tora (1)[b]	chlorotic spots to mottle in new growth, dark vein feathering in older leaves
Chenopodium album (5, 7)	distinct circular yellow spots with pale centers on inoculated leaves
C. amaranticolor	distinct chlorotic or necrotic spots sometimes with red borders on inoculated leaves
Datura stramonium (1, 5, 7, 8)	veinclearing in new growth, epinasty, blistering, leaf distortion
D. metel	do.
Helianthus annuus (7)	sometimes necrotic rings on inoculated leaves
Lycopersicon esculentum (7, 8)	
'Homestead'	mild veinclearing and mottle with leaf crinkle, pronounced epinasty
'Rutgers'	do.
'Rio Grande'	do.
'Chico'	do.
Margaranthus solanaceus	veinclearing, epinasty, reduction in leaf size, chlorosis of interveinal tissues, abscission of flowers
Nicotiana tabacum (1, 2, 5, 7, 8)	
'Kentucky Burley 16'	veinclearing, mild mottle, veinbanding, followed by chlorotic to brown etch designs
N. glutinosa (5, 7)	veinclearing, wavy leaf margins, rugosity
N. repanda (7)	chlorotic spots becoming necrotic flecks, pronounced rugosity
Petunia hybrida (7)	veinclearing, mild mottle, leaf distortion, light spots and streaks in pink flowered varieties
Phacelia campanularia (7)	faint chlorotic mottle, light spots and streaks in dark blue corollas
Physalis floridana (5)	veinclearing, pronounced epinasty, dwarfing, chlorotic mottle, interveinal necrosis
P. peruviana (6, 7)	mild veinclearing, leaf margins turned upward, rugosity, filiform leaf distortion
Solanum elaeagnifolium	faint necrotic flecking on inoculated leaves, rugosity, often no symptoms
Zinnia elegans (7)	veinclearing, mild mottle
Vigna sinensis (7)	no reaction
Phaseolus vulgaris (7)	
'Pinto'	do.
'Black Valentine'	do.
Cucumis sativus (7)	do.
Gypsophila elegans (7)	do.
Phlox drummondii (7)	do.
Malvastrum coromandelianum	do.
Gomphrena globosa (5)	do.
Crotalaria juncea	do.
Antirrhinum majus	no reaction
Chenopodium murale	do.

[a] Numbers in parentheses indicate literature reference to species previously tested by others.

[b] Test plant suggested in correspondence with C. W. Anderson, Gainesville, Florida. Resistant to TMV, CMV, and PYV (C. W. Anderson).

severe filiform leaf distortion, and mottle in *P. peruviana*. Greenleaf (5) suggested *Chenopodium album* as a local-lesion indicator species for TEV. In the present studies, *C. amaranticolor* was a more reliable local-lesion indicator plant.

During a search for natural weed hosts, white horsenettle (*Solanum elaeagnifolium*) was suspected because field infections appeared to originate near patches of this common weed. Symptoms, however, have not been observed in the field nor has the etch virus been isolated from suspicious plants. White horsenettle rarely expressed symptoms after mechanical inoculation but occasionally pinpoint, black, necrotic lesions appeared on inoculated leaves. Such leaves soon abscised; terminal growth was rugose. *Nicotiana repanda* also is a very common weed in south Texas but virus symptoms have not been observed on it in the field. A natural reservoir of the virus is not known.

Whether field infections on pepper and tomato were caused by severe tobacco-etch virus, or mild-etch virus, or both strains is not known. Some isolates failed to cause a rapid wilting in 'Tabasco.' Tabasco plants inoculated with the slow-wilting isolates "protected" against challenge inoculations with the severe, rapid wilting isolates.

In these survey studies, potato Y virus (PYV) was rarely isolated from 'Yolo Wonder' peppers. It was nearly impossible to distinguish symptoms of TEV from those of PYV on pepper. PYV was identified by pronounced veinbanding symptoms in tobacco, severe mottle and rugosity in 'Tabasco,' severe rugose distortion in *Physalis peruviana*, and failure to infect Jimson weed.

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ISOLATION OF A MUTANT STRAIN OF POTATO Y VIRUS[1]

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Resistance to one strain of potato Y virus previously designated PVY-N has been reported in three varieties (P.I. 264281, S.C. 46252, and YRP10) of Capsicum annuum (2, 3). Virus recovery attempts from both mechanically- and graft-inoculated plants of these varieties at various times from 54 to 151 days after inoculation were consistently unsuccessful. It was concluded from genetic studies that a single (homozygous) recessive allele, common to all three varieties, conferred resistance to this virus strain (1, 2, 4).

Plants of P.I. 264281 and S.C. 46252 were discarded after completion of the studies just mentioned. As a means of maintaining the original plant material, some of the plants with graft-inoculated (but symptomless) scions of YRP10 were kept. Approximately a year after these YRP10 scions had been graft-inoculated, symptoms consisting of a distinct systemic mottle were noted in one of them. Based on the responses of differential hosts (Datura stramonium, Capsicum frutescens var. Tabasco, and Nicotiana spp.) to the virus recovered, the conclusion was reached that this YRP10 scion was infected with potato Y virus.

Repeated attempts to infect plants of the varieties P.I. 264281 and S.C. 46252 with the virus from the infected YRP10 scion were unsuccessful although progeny from selfed pollinations of YRP10 were uniformly susceptible. Plants of pepper varieties known to be susceptible to the original strain of potato Y virus (PVY-N) were also susceptible to the isolate from the YRP 10 scion.

For previous studies, potato Y virus cultures had been maintained by frequent transfer in N. tabacum var. Turkish plants. The virus isolate from the YRP10 scion was infectious on Turkish plants, as was the original strain. Inoculum from Turkish plants recently infected with the former induced symptoms in all plants of (known) susceptible varieties. After the virus had been serially transferred three or more times in Turkish, it no longer induced symptoms in YRP10 plants, although it was still infectious on "susceptible" pepper varieties and tobacco species. No comparable alteration of infectivity was noted after numerous serial transfers of this virus, designated PVY-N^{YR}, in YRP10 plants.

Isolation of the mutant virus strain discussed here has been considered to result directly from the selective susceptibility of YRP10 for a spontaneous mutation which occurred in the original virus strain. Since no evidence of variability in infectiousness of PVY-N was noted in previous studies, the mutant virus strain probably developed subsequent to inoculation of YRP 10 scions.

In previous studies, it was determined that the resistance of YRP10 and P.I. 264281 to the original virus was effected by a (homozygous) recessive gene common to both plant types. As demonstrated by the distinct responses of these same plant types to the mutant virus, the "extended graft technique" may offer a means for critical genetic analysis. Realization of this possibility would require that additional specific mutations be obtained with different host-virus systems. This procedure also could be utilized to assess the potential mutability of virus isolates.

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A PINE DISEASE IN NEW ZEALAND RESEMBLING LITTLELEAF

George H. Hepting[1] and F. J. Newhook[2]

Newhook (2) has described a littleleaf-like disease of Monterey pine (*Pinus radiata*) on heavy soils in the Northland of New Zealand. He reported five Phytophthoras (mostly *P. cinnamomi* Rands) associated with the roots of the diseased trees. Having seen littleleaf of shortleaf and loblolly pine in the southern United States, and the relation of *P. cinnamomi* to this disease, Newhook remarked on the similarity of the decline of shelterbelt Monterey pine on clay soil in New Zealand to littleleaf in the United States.

In 1960 Hepting visited New Zealand and examined Monterey pine under a wide variety of conditions, from light pumice soils to the very plastic clays of Northland. One of the heavy clay areas was at Waipoua in the Kauri forest. Here he was shown neighboring pine plantings, several acres in extent, of shortleaf (*Pinus echinata*) planted in 1929, loblolly (*P. taeda*) planted in 1934, longleaf (*P. palustris*) in 1934, and Monterey pine in 1934.

The shortleaf pine had begun to die a few years before, and when seen in 1960 the stand was completely breaking up. The symptoms of short and yellow needles, progressively shortening internodes, and early death were so like littleleaf on shortleaf in the United States that color photographs of the condition in New Zealand and in the United States could not be distinguished from each other by American pathologists working on littleleaf. Occasional loblolly and longleaf pines were similarly affected. In the United States also, shortleaf pine is most seriously affected, loblolly next, and under extreme conditions of heavy, wet soils longleaf will sometimes show symptoms. Opposite the affected shortleaf pine stand at Waipoua was a stand of Monterey pine with the symptoms of littleleaf.

Prior to 1960, Newhook had isolated *P. cinnamomi* from soil samples sent to him from the Kauri nursery less than a mile from the diseased shortleaf pine stand, but he had not seen the latter until Hepting's visit in 1960. Newhook subsequently assayed for Phytophthoras, using Campbell's technique (1), by taking 50 soil samples from each of several sites in the Waipoua area and placing subsamples in apples. *P. cinnamomi* was abundant in the rhizospheres of the dying shortleaf pines (isolated from 39 to 72% of the samples), nearby similarly affected radiata pine (isolated from 75% of the samples), and from nearby sites with indigenous forest (17 to 39 %). *P. cactorum* was also isolated from some soils under nearby native cover.

Littleleaf is a symptom complex of particular severity on shortleaf pine and is associated with wet, heavy soils or poor subsoil drainage and *P. cinnamomi* infection of roots. The declines of this species at Waipoua, New Zealand, and in the southern United States appear very similar. Among the similarities are the lesser attack on loblolly and longleaf pine, first losses after an age of 20 to 30 years, and association with wet, heavy soils in which *P. cinnamomi* is abundant. The entire disease picture of shortleaf pine both in New Zealand and North America is consistent with that of Phytophthora root rots of avocado, pineapple, yew, heath, and many other crops (3).

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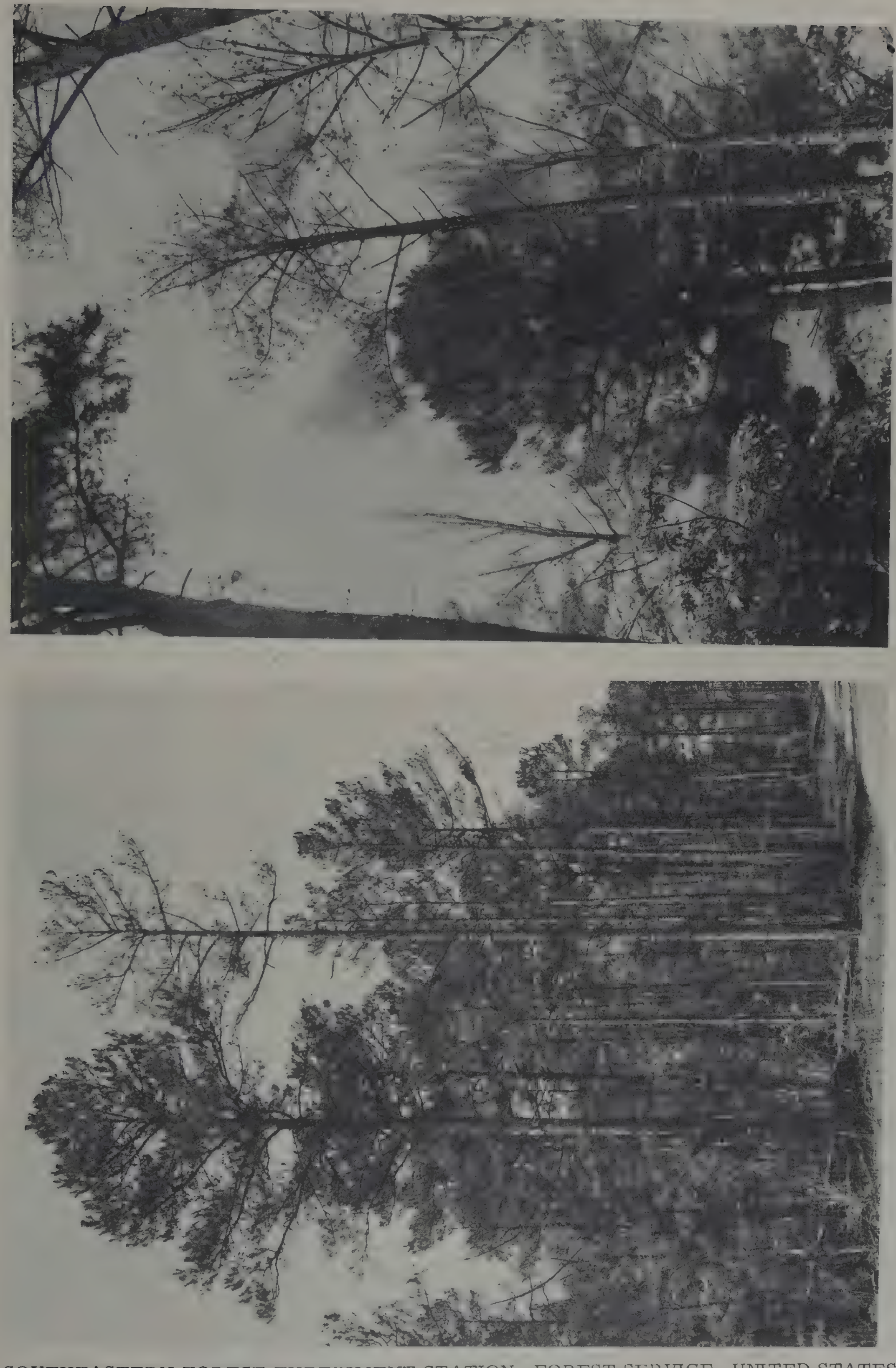

FIGURE 1. Left -- littleleaf and healthy shortleaf pine, Athens, Georgia, U. S. A. Right -- diseased and healthy shortleaf pine, Waipoua State Forest, New Zealand.

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SPECIES OF ROOT-LESION NEMATODES COMMONLY ASSOCIATED WITH ECONOMIC CROPS IN THE DELTA OF THE U.A.R.[1]

Bakir A. Oteifa[2]

Plant-parasitic nematodes have been recognized in the United Arab Republic as an important factor in crop production since 1901 when Preyer (6) first discovered a nematode disease in banana plantations at Alexandria. Subsequently, reports of the occurrence of parasitic nematodes on various host plants were recorded by Fahmy (2), Melchers (4) and Fikry (3). Most of these early records were concerned with the group of root-knot nematodes. As a preliminary step in development of nematode control methods, the author found it necessary to proceed with a general survey study (5).

All samples contained various nematode genera, but this paper presents only the portion of the survey data on occurrence of root-lesion nematodes, genus Pratylenchus Filipjev, 1934, found in association with economic crops grown in the Nile Delta Valley. No attempt has been made to include unidentified species of this genus, but only species included in the monograph by Sher and Allen (7).

MATERIALS AND METHODS

Soil samples of approximately 1 pint were collected from various farms throughout the Delta. Each sample was a composite of scattered borings from a field. Root samples with their soil rhizospheres were also collected. All samples were stored in polyethylene bags and were kept at 4° C until processed. The methods of Christie and Perry (1) were used to separate the nematodes from soil. The Waring Blendor method used by Taylor and Loegering (8) was used to extract nematodes from root tissues. Nematode species recovered were determined from specimens killed in hot water and fixed in 5% formaldehyde.

Percentage occurrence of the genus Pratylenchus was determined by recording the number of times it was identified. The frequency index of each species of Pratylenchus was determined by placing samples in the following categories: 0 = a frequency of no specimens per sample; 1 = 1 to 5; 2 = 6 to 10; 3 = 11 to 15; 4 = 16 to 20; 5 = over 20 specimens per sample.

RESULTS

In the economic crop plants which are grown extensively in the Delta region of the Nile valley, the following ten species of Pratylenchus are commonly found: P. brachyurus (Godfrey, 1929) Filipjev & Stekhoven, 1941; P. coffeae (Zimmermann, 1898) Filipjev & Stekhoven, 1941; P. goodeyi Sher & Allen, 1953; P. minyus Sher & Allen 1953; P. penetrans (Cobb, 1917) Filipjev & Stekhoven, 1941; P. pratensis (de Man, 1880) Filipjev, 1936; P. scribneri Steiner, 1934; P. thornei Sher & Allen, 1953; P. vulnus Allen & Jensen, 1951 and P. zeae Graham, 1951. Table 1 presents the list of crop plants with the number of samples examined and the percentage occurrence of the genus Pratylenchus. Its general recovery from cotton, corn, peanuts, banana, potato, strawberry, tomato and watermelon indicates that it is an important biological factor in the deterioration of such crops. Its occurrence, on the other hand, in samples from all crops examined indicates that this genus is not restricted to specific plant families or even to specific ecological conditions. Species of the genus, however, seem to be better adapted to some hosts than to others, emphasizing the importance of species determination.

Table 2 shows the relative frequency of species associated with various crops and a frequency index for each species. P. brachyurus and P. penetrans, with frequency indices of 17.6 and 13.6 respectively, are the most common species in the Delta region of the Nile valley. P. coffeae, P. thornei, P. minyus and P. zeae are next, while P. vulnus, P. goodeyi, P. pratensis, and P. scribneri were found least often.

The table also indicates that some species have a limited host range while others have a wider host range. P. brachyurus, for example, is associated more with field and vegetable crops than with fruit crops. On the other hand, P. penetrans is associated more with fruit and

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Table 1. General occurrence of root-lesion nematodes associated with crops grown in the Nile Delta Valley in 1961.

Common name	Scientific name and variety	No. samples examined	Occurrence of Pratylenchus (%)
Field Crops			
Broadbean	*Vicia faba* var. Balady[a]	25	36.0
Clover[b]	*Trifolium alexandrinum* var. Balady	50	35.3
Corn	*Zea mays* var. Nabelgamal	115	61.8
Cotton	*Gossypium barbadense* var. Giza 30	223	42.6
Peanut	*Arachis hypogaea* var. Procumbens	48	81.2
Rice	*Oryza sativa* var. Balady	35	36.1
Wheat	*Triticum pyramidale* var. Balady	25	48.5
Wheat	*Triticum vulgare* var. Hindy	20	50.2
Total		540 [541]	48.9
Fruit Crops			
Banana	*Musa cavendishii* var. Hindy	50	81.6
Banana	*Musa sapientum* var. Maghraby	70	78.5
Grape	*Vitis vinifera* var. Roomy	75	56.6
Lime	*Citrus aurantifolia* var. Balady	69	12.4
Mandarin	*Citrus reticulata* var. Balady	51	11.7
Mango	*Mangifera indica* var. Hindy	75	10.4
Orange	*Citrus sinensis* var. Balady	105	5.3
Orange (sweet)	*Citrus sinensis* var. Soukary	80	19.8
Peach	*Prunus persica* var. Balady	115	85.2
Total		690	40.7
Vegetable Crops			
Cowpea	*Vigna sinensis* var. Azmerly	162	82.7
Cucumber	*Cucumis sativus* var. Balady	24	66.7
Okra	*Hibiscus esculentus* var. Balady	38	73.6
Pea	*Pisum sativum* var. Balady	30	23.3
Potato	*Solanum tuberosum* var. Alpha	93	76.3
Strawberry	*Fragaria* sp. var. Balady	85	65.6
Stringbean	*Phaseolus vulgaris* var. Balady	35	40.0
Sweetpotato	*Ipomoea batatas* var. Mabrouka	49	36.5
Tomato	*Lycopersicum esculentum* var. Pritchard	126	80.8
Watermelon	*Citrullus vulgaris* var. Balady	95	89.5
Total		737	73.0
Total Crops		1967 [1968]	54.5

[a] Balady means "local" variety.

[b] Egyptian clover known as "Barseem."

vegetable crops than with field crops. A relatively high frequency of *P. brachyurus* occurs with cotton, peanuts and strawberry crops, while such high frequency for *P. penetrans* occurs with peach, tomato and watermelon. *P. coffeae* is found in high frequency in association with banana and cowpea crops. *P. goodeyi* is confined more to banana crops despite its occurrence in the soils of broadbeans, rice, cowpeas, strawberry and watermelon. *P. minyus* is more prevalent in corn, grape, peach, cowpeas, potato and watermelon fields. *P. pratensis* was seldom found in large numbers despite its recovery from almost half of the crops examined. *P. scribneri* seems to be restricted to very few crops. Its recovery from potato and sweetpotato, in particular, is of interest. *P. thornei* was often recovered from cowpeas, corn, and tomato fields. *P. vulnus* seems to be more selective, occurring on particular hosts such as grapes, peaches, strawberries and watermelons. *P. zeae* is undoubtedly a corn nematode despite its fluctuating frequency among other crops.

Table 2. Degree and frequency index of Pratylenchus species associated with crops grown in the Nile Delta Valley in 1961.

Crops	Relative frequency of Pratylenchus species									
	br.[b]	co.	go.	mi.	pe.	pr.	sc.	th.	vu.	ze.
Field										
Broadbean	2	1	1	0	0	0	0	0	0	1
Clover	2	0	0	1	1	1	0	0	1	0
Corn	2	0	0	3	1	1	0	4	0	5
Cotton	5	0	0	1	1	0	0	1	0	3
Peanut	5	1	0	1	2	0	0	1	0	0
Rice	1	0	1	1	2	1	0	1	0	1
Wheat (Balady)	0	0	0	1	1	0	0	1	0	2
Wheat (Hindy)	0	0	0	1	2	0	0	1	0	1
F.I.[a]	35.2	0.3	0.2	3.4	2.1	0.2	0.0	4.2	0.2	12.5
Fruit										
Banana (Hindy)	1	4	4	1	1	1	0	0	1	0
Banana (Maghraby)	2	5	3	1	1	1	0	0	1	0
Grape	1	0	0	3	4	1	0	0	3	0
Lime	1	0	0	1	1	0	0	0	1	0
Mandarin	1	0	0	1	1	0	0	0	1	0
Mango	1	0	0	1	1	0	0	0	1	1
Orange (Balady)	0	0	0	1	1	0	0	0	0	0
Orange (Soukary)	1	1	0	1	2	1	0	0	1	0
Peach	1	0	0	3	5	1	0	1	3	1
F.I	1.8	11.2	6.9	5.6	22.6	2.7	0.0	0.1	5.2	0.1
Vegetable										
Cowpea	4	5	1	3	2	1	1	5	1	2
Cucumber	1	0	0	0	1	2	0	0	1	0
Okra	4	0	0	0	1	0	0	1	0	2
Pea	1	1	0	0	1	0	0	1	0	0
Potato	4	3	0	3	3	1	2	2	0	0
Strawberry	5	0	1	1	3	2	1	0	3	0
Sweetpotato	3	0	0	0	1	0	2	1	0	1
Tomato	3	2	0	1	5	2	0	4	0	1
Watermelon	2	1	1	3	5	1	1	0	2	1
F.I.	10.3	4.5	0.1	2.4	10.1	1.2	0.7	6.3	1.6	0.8
General F.I.	17.6	6.2	1.5	3.6	13.6	1.3	0.4	5.6	2.1	3.5

[a] Frequency index for all crops was calculated by employing the formula $P = \frac{(nxv) \times 100}{6 N}$; where P = frequency index; n = number of times the species was identified in each frequency category; v = numerical values of frequency categories; and N = total number of times the species was identified.

[b] br. brachyurus; co. coffeae; go. goodeyi; mi. minyus; pe. penetrans; pr. pratensis; sc. scribneri; th. thornei; vu. vulnus; ze. zeae

DISCUSSION

Root-lesion nematodes probably constitute a major pest problem in the agricultural economy of United Arab Republic. Wherever these nematodes were found in cultivated fields, they were associated with crop decline even though soil moisture was adequate and good management practices were followed. In most instances, the environmental factors which control their prevalence are not known. They are frequently found in vegetable crops in association with microorganisms of root rot and wilt diseases and apparently have an important role in the complex problem. Since the general agricultural system in the Delta region of U. A. R. involves the cultivation of at least two successive crops a year, it would seem to be important to determine the host range of each nematode species before attempting recommendation of rotation control programs.

Although the present study was restricted to the determination of the species frequency of Pratylenchus species associated with various crops, several observations made during the survey are of interest.

1. A possibility exists that mixed populations might be segregated through host preference. Confirmation will require a series of tests on large numbers of host plants under controlled conditions.
2. To avoid the hazards of buildup and spread in crop rotation programs and in the selection of cover crops for orchards, the determination of prevalent nematode species is of great importance.
3. The common occurrence of known root-lesion nematode species in U. A. R. suggests that their geographical distribution is very wide.

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SPORULATION, GERMINATION, AND EARLY GROWTH OF ECHINODONTIUM TINCTORIUM

Orson K. Miller, Jr.[1]

Abstract

The Indian paint fungus was found to sporulate shortly after cessation of typical winter conditions. Sporulation was light, and the basidiospores did not germinate after 70 hours' incubation at 25° C. However, when sporophores were brought into the laboratory and sections of the teeth mounted in various solutions, germination was observed. In the first 70 hours of growth, septa were produced and branched hyphae had grown as much as 133 microns. Presents details on growth of the germ tube and the viability of the basidiospores in different solutions. Evidence obtained from this experiment indicates that the basidiospores are nutritionally independent but subject to desiccation.

The Indian paint fungus, Echinodontium tinctorium Ell. & Ev., is a major cause of heart rot in several western conifers. It causes abundant decay in western hemlock (Tsuga heterophylla) and grand fir (Abies grandis) in the forests of northern Idaho and eastern Washington. Several studies on the amount of decay caused by this fungus have been published (1, 2, 3, 4, 7), its growth rate (5), and its distribution (6), but little has been done in defining its life cycle. In culture, the fungus produces only chlamydospores; therefore, it is assumed that the basidiospores provide the most frequent means by which the fungus infects host trees. However, previous attempts to collect and germinate the basidiospores have been unsuccessful. This paper reports successful germination of the basidiospores, their viability, and early growth of their germ tubes.

METHODS

Glass slide spore traps were prepared by placing a drop of a defined medium[2] in the center of each clean sterile slide. Small trays to hold the slides were fashioned from aluminum foil, and ordinary masking tape was used to hold the trays in place beneath in situ sporophores. The slides were carried into the field in sterilized slide boxes, placed in the trays for about 1 hour, and then removed and placed in an empty sterile box for transport back to the laboratory.

Other sporophores were cut from trees, placed in polyethylene bags, and stored at 9° C in the laboratory until used. Just prior to study, these sporophores were kept at room temperature for approximately 48 hours. An individual tooth was severed from the hymenium, and thin cross sections were cut with a razor blade through the lighter gray upper portion of the tooth but not through the reddish-brown apices that have little or no mature hymenial elements. The sections were mounted in the following solutions: 10% peptone, 10% malt, 1% thiamin, distilled norite water, and 1% yeast extract. Cover slips were placed over the mounted material, and each slide was placed in a Petri dish damp chamber. These Petri dishes were stored at room temperature (approximately 25°). This method permits observation of the same section over a period of days quickly and without disturbing the spores. The damp chamber keeps the sections moist, and the same spore or group of spores can be relocated on successive days.

RESULTS

Five sporophores, of many observed, were selected for study on grand fir trees located near Benton Creek and Canyon Creek on the Priest River Experimental Forest in northern Idaho. Traps set up on each sporophore were left in place for approximately 1 hour in midday on April 25 and 26, 1962. Subsequent study of the slides revealed that two of the five sporophores were producing, but production was light.

Maximum temperatures on April 25 and 26 were 54° and 53° F and the minima were 30° and 25°, respectively. A trace of precipitation occurred on the 26th, and the relative humidity

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[2] Medium consisted of: 20 g sucrose, 15 g agar, 20 ml Vogel's solution containing trace elements, 2.0 ml yeast extract, 4 ml thiamin per liter of distilled water.

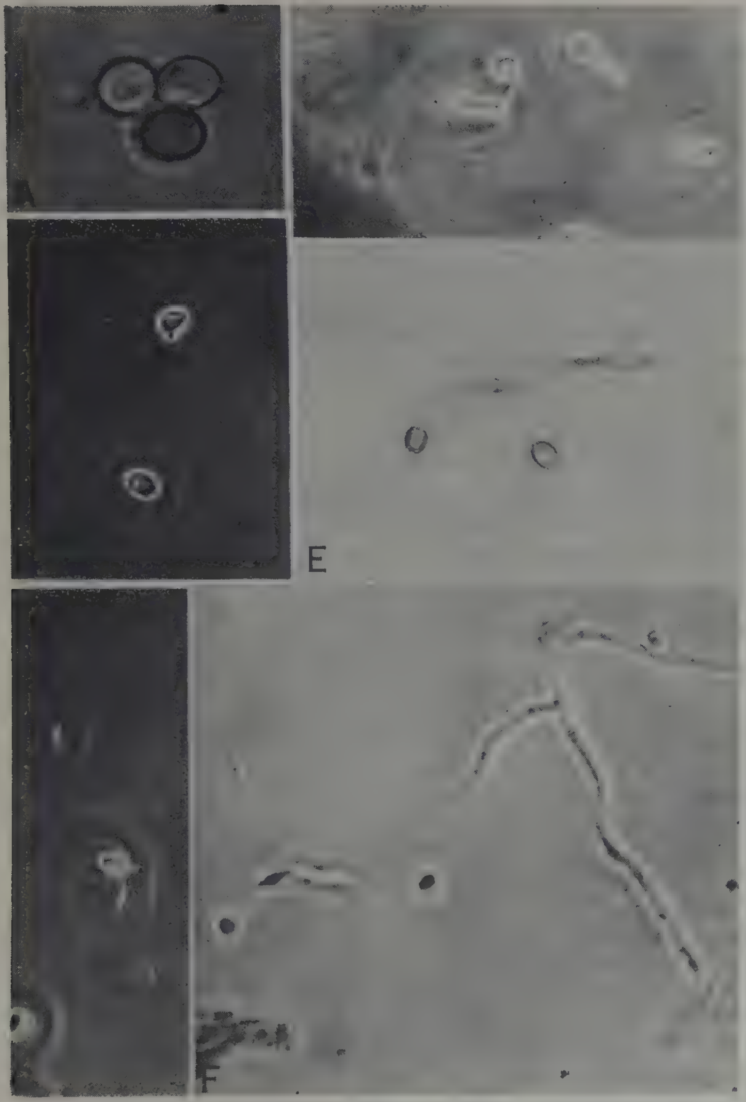

FIGURE 1. Germination and early growth of the germ tube of Echinodontium tinctorium. A -- Twenty-two hours after mounting in liquid media, maximum length of germ tubes was 4.5μ (spores are 5.5-7.0 x 3.5-4.5μ). B -- Average length of germ tubes after 22 hours was 1 or 2μ. C -- After 44 hours germ tubes were about 8μ long. D -- Although some tubes were as long as 12μ, no septa were found after 44 hours. E -- After 70 hours, septa were visible (germ tube 59μ long). F -- Also after 70 hours, some of the more advanced germinating spores were producing multiseptate, branched hyphae. Magnification approx. X480, except A, approx. X1080.

fluctuated between 42 and 100%. Patches of snow were still on the ground in the study areas at about 2600 feet elevation while snow cover was almost solid above 3500 feet, although prior to the 25th temperatures were in the high sixties and low seventies. However, this warm weather prior to the 25th was the first of the season following typical winter conditions.

Periodic sampling of basidiospore production in this area indicates that sporulation commences very soon after temperatures rise above freezing, but the relative amount of spore fall and the ability of these early spores to survive and germinate are not known. Both of these aspects of the relation of early sporulation to subsequent infection are now being studied. None of the spores collected on the slides in the field germinated after 70 hours of incubation at 25° C.

One of the study sporophores producing spores in situ and several additional ones were brought into the laboratory and stored as previously described. Five days later, sections of the hymenium of one sporophore were mounted on slides in the different solutions (see "Methods"). At that time, several sections were mounted and many spores observed, but none had germinated. Immature spores on the sterigmata indicated that sporulation was probably occurring on the sporophores within the polyethylene bags. The first set of sections mounted in solutions of 10% peptone, 10% malt, 1% thiamin, distilled norite water, and 1% yeast extract were studied 22 hours after the mounts were made. Initial germination was observed in mounts of all solutions tried. This is the first account, to the writer's knowledge, of observed germination of basidiospores of E. tinctorium.

After 22 hours, the germ tubes were 1 or 2 to several microns long (Fig. 1 A, B). After 44 hours, many more spores had germinated, and germ tubes up to 12μ long were observed (Fig. 1 C, D). At the end of 70 hours, germ tubes were common, the first septa were visible (Fig. 1 E), and branching hyphae from the more advanced germinating spores could be seen (Fig. 1 F). Growth of the germ tube and subsequent hyphal growth in the more advanced spores ranged from 45 to 133μ in length. No clamp connections were observed on any of the septate hyphae arising from the basidiospores, although they occurred at every septum on new hyphae which grew from the cross sections of the sporophore teeth during the experiment. No detectable increase in the number of spores was observed after 22 hours; this indicated that sporulation had not continued to any great degree after the sections were cut and mounted. Therefore, after 70 hours, spores were counted to obtain a preliminary estimate of their viability under the experimental conditions employed. More than 150 spores were counted in each sample. Spore germination on 10% malt, 1% yeast extract, and 10% peptone and distilled norite water ranged from 5.7 to 7.6%; but less than 1% of the spores in 1% thiamin had germinated during this same period.

CONCLUSIONS

Results of this study indicate: 1) that the delicate thin-walled basidiospores of E. tinctorium are capable of rapid germination and early growth; and 2) that they have rather stringent moisture requirements for germination and may even require an aqueous medium.

The addition of complex media did not increase viability of the basidiospores above that in distilled norite water. This indicates that they are nutritionally independent and that desiccation probably is the most important factor in reducing their viability. A 1% thiamin solution appeared to inhibit germination.

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INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION, FOREST SERVICE,
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RESISTANCE TO YELLOW DWARF OF OATS IN WASHINGTON[1]

G. W. Bruehl, V. D. Damsteegt, H. M. Austenson and P. C. Crandall

Abstract

The oat varieties and species of the active oat collections of the United States Department of Agriculture were tested for tolerance to the barley yellow dwarf virus in Washington in the field and greenhouse, 1959-1962. Evidence of important virus strain-oat variety reactions is presented. C. I. Nos. 1580, 1837, 2336, 2871, 2873, 2874, 2876, 4207, 4548, 4657, 4664, 4718, 5068, 5145, 6975, 6979, 7295, and P. I. Nos. 180942, 182495, 182500, and 183985 are recommended for trial as possible sources of tolerance to yellow dwarf in oats.

Yellow dwarf (BYDV) or red leaf is usually the most serious disease of oats in western Washington, and is frequently serious on late-seeded spring oats in the eastern part of the State. A search for resistance in oats to this disease was made as a first step toward reducing disease losses. Field screening during natural epidemics was employed in 1959-1961 inclusive, followed by a greenhouse test of the selections most promising in the field trials.

1959

In the fall of 1958, a mixture of winter oats, wheat and barley was seeded in strips at the Southwestern Washington Experiment Station, Vancouver, to attract and nurture aphids. The winter of 1958-1959 was mild and aphids were abundant in the strips of winter grain by early spring and the cereals showed ample evidence of yellow dwarf. Between April 7-9, the Active World Oat Collection (3587 varieties) and the Oat Species Collection (148 entries) of the United States Department of Agriculture were seeded between the infested strips of winter cereals in duplicate short rows 1 foot apart. Albion (C. I. 729), a common oat, and Saia (C. I. 4639), an *Avena strigosa* selection, both reported fairly tolerant by Endo (6), were seeded in every eleventh and twelfth row to provide standards for comparison.

Apple grain (*Rhopalosiphum fitchii* (Sanderson)), English grain (*Macrosiphum granarium* (Kirby)), and rose grass (*M. dirhodum* (Walker)) aphids moved rapidly into the oats as the seedlings emerged. Infection by BYDV was 100% and comparative tolerance to the disease was measured as the oats approached maturity. Evaluation was based on vigor, discoloration, tillering and sterility. Four hundred forty-five varieties in the Active World Oat Collection were judged equal or superior to Albion. These were harvested for further testing.

1960

Each of the 445 oats selected in 1959 plus all entries of the Oat Species Collection were seeded in triplicate rows 8 feet long in early April at Vancouver. A single row was also seeded at Puyallup, Washington. Shasta (C. I. 3976) served as the standard. In 1960 aphids did not enter the oat nurseries in appreciable numbers until after tillering. Nevertheless, by the time the oats approached maturity, 100% infection had been achieved and discoloration and sterility developed to a degree permitting selection of varieties for tolerance. Seventy varieties were harvested for further screening. As the common oats apparently possessed resistance equal to that found in any of the entries in the Oat Species Collection, the latter were dropped from further tests.

1961

Two nurseries, composed of the 70 varieties selected in 1960 for further testing plus several miscellaneous varieties, were seeded in western Washington in early April, one at Vancouver with three replicates, and one at Puyallup with two replicates. Each replicate was a 3-row plot, with rows 18 inches long. This provided a wealth of growth upon which to evaluate tolerance to yellow dwarf. Among the miscellaneous varieties included in the test were Albion (C. I. Nos. 729, 1050, 3460), Beedee, Columbia, Newton, and Putnam, all of which had shown useful degrees of tolerance in the midwestern epidemic of 1959 (2, 5, 7, 10, 11, 12). Apple grain,

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[1] Scientific Paper No. 2248, Washington Agricultural Experiment Stations, Project 1546.

Table 1. Response of some oat varieties to yellow dwarf.

C. I. or P. I. No.	ENTRY Name or pedigree	Average no. of green leaves, July 5-6, 1961, Vancouver-Puyallup	Yield rating Puyallup, 1961	% green weight diseased/ healthy Walla Walla strain
729	Albion	0.66	301	61[a]
1012	White Kherson (Olds Albion)	0.62	357	63
1050	Albion	0.34	302	64
1320	Victory	1.48	256	11
1580*[b]	Black Tartarian	2.42	443	56
1837*	Gray Moor (late)[c]	2.34	295	71
2336*	Cowra No. 35	2.06	469	62
2820	Columbia	0.00	104	20
2871*	Kherson	2.16	492	56
2873*	Minrus	2.32	430	51
2874*	Minnesota No. II-22-220	2.86	469	33
2876*	Richland x Fulghum	2.52	428	62
3460	Albion	0.78	347	60
3976	Shasta	1.70	482	11
4181	Overland	0.04	299	7
4207*	Pioneer (late)	3.50	409	59
4548*	Raleigh (late)	3.04	451	54
4657*	Arlington (late)	2.20	277	73
4664*	Red Rustproof type oat	2.80	451	71
4718*	(D69 x Bond) x Iowa sel. 8	2.54	411	84
5068*		2.20	388	76
5145*	Fultex x (RRP x Victoria-Richland) (late)	2.60	375	55
6642	Newton	0.28	227	50
6701	Clintland	0.46	196	8
6752	Beedee	0.54	243	30
6927	Putnam	0.28	93	--[d]
6975*	Fultex x Iogold-Bond (late)	1.56	366	43
6979*	Fulgrain Strain 6 x Fulkedeer (late)	2.60	375	54
7010	*Avena strigosa*	--	--	70
7234	Clintland 60	0.64	223	7
7295*	Crater (late)	2.80	391	45
180942*		1.82	458	52
182495*	Narym 0222-21, S-7	1.56	319	95
182500*	Sakhalin Baikal 28412 S-15	1.78	551	35
183985*		2.56	555	26
	Craig x Alamo, Cornell Sel. 51	2.00	443	23
	Curt	0.56	230	--

[a] Albion, used as a check, was present in this test seven times. 61% is the average of 30, 43, 63, 64, 64, 70, 72.

[b] Those indicated by an asterisk are suggested for further trial.

[c] Late maturity indicates a probable lack of adaptation to spring seeding, and the yield score may be relatively low.

[d] Not tested.

English grain, and rose grass aphids were abundant in early spring and also constantly present throughout the growing period. Infection was 100% and uniform. Two leaf readings were made, one in early jointing stage (June 13-14) and the other after the early varieties had headed but before natural senescence had affected the leaves (July 5-6). The number of expanded leaves per plant that were still green or otherwise not obviously affected by yellow dwarf was estimated in each plot on each date and a leaf rating indicating the ability to maintain a green color was calculated. Birds damaged the Vancouver plots; consequently the yield scores presented (Table

1) are based on a total of 24 feet of harvested row per variety at Puyallup. The reactions of the varieties as judged visually at the two stations were essentially the same and are therefore presented collectively (Table 1).

GREENHOUSE TEST, 1962

In February-March, most of the oat varieties under study were seeded in 4-inch clay pots in the greenhouse at Pullman, Washington. On the average, each variety was represented by 10 pots (three plants per pot), five inoculated and five uninoculated. In this greenhouse test a virulent isolate of BYDV derived from winter barley growing near Walla Walla in eastern Washington and the English and apple grain aphids were used. The viruliferous aphids were placed on the oats in the 1 to 2 leaf stage, allowed to feed for 24 hours, and then removed by fumigation. The inoculated and check plants were grown under identical conditions on the same greenhouse benches with ample watering and fertilization, until the growth approached the limit possible in small pots (Figs. 1 and 2) without introducing a stunting effect. At that time (mid-jointing) the plants were cut near the ground and the green weights obtained. The ratios of mean green weights, inoculated/check, expressed as percent (Table 1), are considered a measure of tolerance to the Walla Walla isolate of the virus.

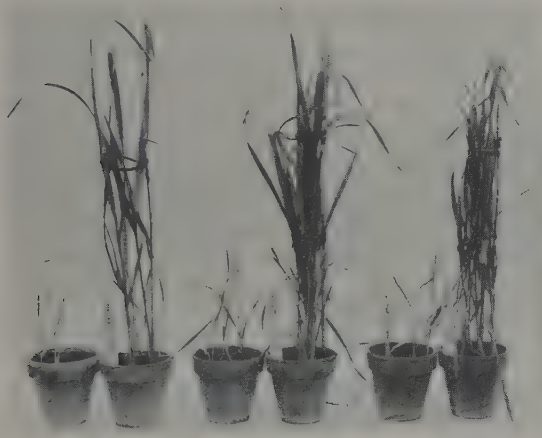

FIGURE 1. Oats grown in the greenhouse, inoculated and the check (left- and right-hand member of each pair respectively), susceptible to the Walla Walla strain of BYDV. Overland (left) and Ajax (center) were susceptible in the field in western Washington in three successive years; Shasta (right) was somewhat tolerant all three test seasons.

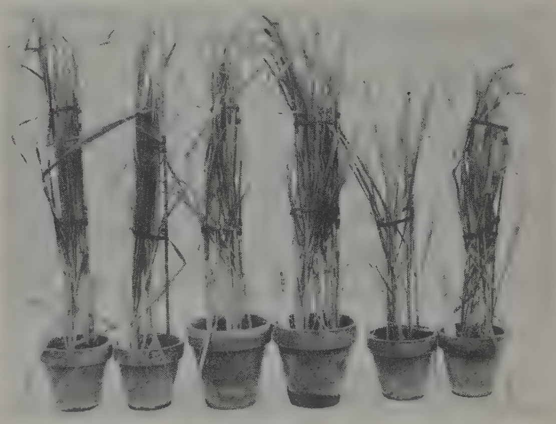

FIGURE 2. Calcutta (left pair), Gray Moor (center pair) and Albion - C. I. 729 (right pair) inoculated (left member of each pair) and check (right member of each pair) showing reaction to the Walla Walla strain of BYDV in the greenhouse. Calcutta and Gray Moor were superior to Albion in the field in western Washington for three successive seasons.

DISCUSSION

Over 3000 of the oat varieties tested in 1959 were so susceptible to BYDV that further consideration of them with respect to the disease was pointless. Although Saia, most of the *A. strigosa* entries, and several representatives of other *Avena* spp. possessed considerable tolerance, observations in the field in 1959 and 1960 indicated that they were of no more value than the best cultivated varieties of *A. sativa* and *A. byzantina*, and they were dropped. This observation is in accord with that of Smith (13) who found Saia (186606) to be partially susceptible to a New York strain of BYDV.

Albion (C. I. 729), selected by Endo (6) as the best common oat and used to advantage in breeding resistant varieties (7), was not outstanding in any of the field trials in western Washington. On the other hand, it did not fail under our most adverse conditions, and when exposed to the Walla Walla isolate (Table 1) in the greenhouse it was among the best.

Shasta was good in the field in all three test years, yet in the greenhouse trial it was very susceptible (green weight inoculated/healthy = .11). Victory was good in field tests in 1959 and 1960, and good in Oregon in 1959 (9), poor in Washington in 1961, and very susceptible in our greenhouse trial (inoculated/healthy = .11). Beedee, Columbia, Newton and Putnam, all tolerant in the midwest epidemic of 1959, were all poor in the western Washington tests in 1961 (4). They were partially tolerant to the Walla Walla isolate in the greenhouse. These and other observations (3, 13) indicate important strain-variety interactions.

There is some evidence that certain varieties possibly possess tolerance to a wide range of strains. Kanota is of some value in California (8, 14), Kansas (12) and Washington (3). Arny (1) selected C. I. Nos. 1012, 1050, 1364, 1963, 4664, 6668, and P. I. No. 180942 as of some value in Wisconsin. The test results reported here corroborate his findings. Additional tests in other regions with the more promising oats listed in Table 1 might reveal that some of them have tolerance to a wide range of strains.

Correlations between yield, early leaf readings, late leaf readings and the leaf readings of both dates combined were made on the 85 varieties in the field plots of 1961. The highest correlation was between the late leaf score and yield (r = .61). This latter correlation is considered high, since it made no allowance for differences in tillering or sterility, and also because several of the greenest varieties were at least partially winter types, and therefore yielded relatively little. It seems probable that a single rating of a nursery near the end of the vegetative period might give a rating of varieties at least adequate for initial screening of large populations.

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THE INCIDENCE OF NECROTIC RING SPOT AND SOUR CHERRY YELLOWS VIRUSES IN COMMERCIAL MAZZARD AND MAHALEB CHERRY ROOTSTOCKS[1]

R. M. Gilmer and L. R. Kamalsky

Summary

The incidence of sour cherry yellows and necrotic ring spot viruses in commercial mazzard and mahaleb seedlings was determined by the cucumber-Butternut squash-Tithonia indexing technique. Virus incidence in mahaleb seedlings was low (0 to 4% in the various lots tested), but incidence in mazzard seedlings was quite high, ranging from 9 to 33%. Of 186 isolates identified from both mazzard and mahaleb seedlings, 170 (91%) were sour cherry yellows virus.

New York nurserymen annually plant 400,000 to 600,000 mazzard (*Prunus avium*) and mahaleb (*P. mahaleb*) seedlings as rootstocks for nursery trees of sweet or sour cherry. In recent years there has been a tendency toward increased use of locally-grown mahaleb seedlings, but well over half of the 425,000 mahaleb and practically all of the 80,000 mazzard seedlings planted in 1962 were imported into New York, largely from the Pacific Coast.

The New York nursery certification program for the control of stone fruit virus diseases requires that nursery trees eligible for certification be propagated from scionwood not more than one propagation removed from indexed foundation trees. Because of this requirement, practically all of the virus contamination in certified nursery trees is derived from infected rootstocks. This report deals with the current incidence of necrotic ring spot (NRSV) and sour cherry yellows (SCYV) viruses in commercially available seedling mazzard or mahaleb cherry rootstocks.

MATERIALS AND METHODS

During the period February-May 1962, samples were collected from commercial seedlings purchased by commercial nurserymen from sources both within and outside of New York. Budwood sections 4 to 6 inches long were removed from seedlings with a pruning shears. These excised dormant sections were partially immersed in tap water and forced in the greenhouse. As young leaf tissue developed on each excised section, it was removed and triturated in a small amount of .06 M K_2HPO_4 buffer. The resulting foliage extract was rubbed gently on the cotyledons of four cucumber plants (var. Ohio MR-17) previously dusted with 400-mesh carborundum.

If symptoms of virus infection developed on inoculated cucumber plants, the seedling rootstock that supplied the inoculum was scored as infected. Transfers of virus cultures from infected cucumber plants to seedlings of Butternut squash and *Tithonia speciosa* were attempted in most cases so that the virus could be identified (1).

Presumably healthy mahaleb and mazzard seedlings -- those that did not induce infection in cucumber -- were not reindexed, although previous experience with the cucumber indexing technique demonstrated that the method was only about 90% accurate (2). Thus, the data to be presented probably indicate a virus incidence lower than was actually present.

RESULTS

A total of 1355 mazzard seedlings from three suppliers and 1124 mahaleb seedlings from five suppliers was indexed on cucumber. Of these, 250 mazzard (18% of those tested) and 32 mahaleb (3% of those tested) seedlings induced a virus reaction on cucumber indicators. Detailed data on the indexing of individual lots are supplied in Table 1.

Only 157 virus isolates from mazzard and 29 virus isolates from mahaleb were transferred from cucumber to Butternut squash. Data on the identity of these isolates are presented in Table 2. The remaining isolates (93 from mazzard and 3 from mahaleb) were not identified, largely because attempted transfers to squash plants from cucumber were not successful. Many of these unidentified isolates were undoubtedly SCYV, which is often difficult to transfer even

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[1] Approved by the Director as Journal Paper No. 1301, New York State Agricultural Experiment Station, Geneva.

Table 1. Incidence of virus infections detected by cucumber indicators in commercial mazzard and mahaleb rootstocks, 1962.

Source and nursery		Origin	No. of rootstocks indexed	No. of rootstocks infected	% infected
Mazzard	A	Pacific Coast	490	161	33
	B	Pacific Coast	485	55	11
	C	Pacific Coast	380	34	9
Totals			1355	250	18
Mahaleb	A	Pacific Coast	41	0	-
	B	Pacific Coast	97	0	-
	C	Pacific Coast	517	23	4
	D	Mid-West	50	0	-
	E	New York	419	9	2
Totals			1124	32	3

Table 2. Incidence of NRSV and SCYV in isolates identified.

Source and nursery		No. isolates identified	No. isolates of SCYV	No. isolates of NRSV
Mazzard	A	105	103	2
Mazzard	B	34	26	8
Mazzard	C	18	16	2
Mahaleb	C	21	18	3
Mahaleb	E	8	7	1
Totals		186	170 (91%)	16 (9%)

under optimum conditions. The proportions of SCYV and NRSV in the unidentified isolates were probably quite similar to those found in isolates that were identified.

No instances of doubly-infected seedlings, those containing both NRSV and SCYV, were found.

DISCUSSION

It is evident from the data presented that the low level of incidence of NRSV and SCYV in mahaleb seedlings indexed (about 3%) does not represent any serious threat to certification of cherry nursery trees in New York. Because cherry buds are less likely to unite with diseased rootstocks (2) and because many of the infected trees can be eliminated by roguing in the nursery, cherry trees on mahaleb rootstocks, if propagated from authorized scionwood, almost invariably meet certification standards.

The present low incidence in mahaleb seedlings represents a marked improvement over the virus status of commercial mahaleb seedlings in 1957, when the virus incidence in the various lots tested ranged from 9 to 18% (2). About 85% of the cherry nursery trees produced in New York are propagated on mahaleb rootstocks, so this diminution in virus levels in mahaleb rootstocks is very encouraging.

However, virus levels in commercial mazzard seedlings, which ranged from 9 to 33% in the various lots tested, are intolerable from the standpoint of certification, since it is doubtful that even very heavy roguing could reduce the incidence of SCYV to the level required by certification standards. Cherries on mazzard rootstocks represent a comparatively small proportion of New York nursery production (15% or less), but there is a continued steady demand for such trees because mazzard rootstocks are better adapted to heavy soils than are mahaleb rootstocks. It is also quite likely that the recent trend toward mechanical harvesting of cherries will increase the demand for trees on mazzard rootstocks.

New York nurserymen have long considered mazzard a more difficult stock on which to propagate than mahaleb, and trees on mazzard often command a 5 to 10 cent premium because of the generally poorer tree stands on mazzard rootstocks. Much of this difficulty in propagating on mazzard undoubtedly results from the high virus incidence present in commercial mazzard rootstocks and the concomitant reduction in bud-take.

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A SURVEY OF PLANT-PARASITIC NEMATODES IN STRAWBERRY FIELDS OF NOVA SCOTIA[1]

Muhammed Waseem and Donald L. Craig

Summary

Nematodes were isolated from rhizosphere samples collected from 49 fruiting strawberry fields in Nova Scotia. Recognized plant-parasitic forms as well as those of unknown feeding habits belonging to the order Tylenchida were identified.

Pratylenchus spp. were the most widespread, occurring in 90% of the fields sampled. Xiphinema americanum was found in 37% of the fields. Whenever large populations of either Pratylenchus penetrans or X. americanum were found the strawberry plants were reduced in vigor. Tylenchus, Psilenchus, Ditylenchus, Tylenchorhynchus, Neotylenchus, Helicotylenchus, Rotylenchus, Paratylenchus, Aphelenchoides and Aphelenchus were commonly found. Specimens of Hexatylus, Hemicycliophora, Criconemoides and Longidorus were found only rarely.

INTRODUCTION

Plant-parasitic nematodes have been recognized as one factor limiting growth and production of strawberry plants both in Europe and many regions of North America (1, 2, 3, 4, 14, 23). They have also been found in strawberry fields in Nova Scotia (9, 10) but, the extent of their occurrence and the amount of destruction caused by them were unknown. The present investigation was undertaken in an attempt to identify those species of plant-parasitic nematodes present in Nova Scotia strawberry fields and to appraise their economic importance.

PROCEDURE

Rhizosphere samples were collected from 49 different strawberry fields in Kings, Hants, Annapolis, Digby and Lunenburg counties of the province of Nova Scotia (Fig. 1). The samples were all from fruiting fields and were collected between May and August 1961. Thirty-nine of the sampled fields were selected at random; the remaining ten fields were known to be diseased. Sampling sites included fields exhibiting 1) excellent growth, 2) poor growth, and 3) patchiness. In fields exhibiting patchiness duplicate samples were taken, one set from the non-vigorous plants and the other from comparatively healthy ones. In all cases, samples 125 to 150 mm deep and 18 mm in diameter were withdrawn. The nematodes were extracted from a 500-cc subsample following the technique of Christie and Perry (6).

The water containing the extracted nematodes was poured into a Syracuse watch glass with subdivisions ruled on the inside as described by Cobb (7). The generic counts were made using 40X magnification of a stereoscopic microscope and the specific identifications under a compound research microscope. For this purpose the nematodes were relaxed by warming them to approximately 55° C and mounting in TAF fixative (8).

RESULTS AND DISCUSSION

Table 1 presents the frequency of occurrence of plant-parasitic nematodes in 49 fields. Most frequently found were the specimens of the genera Pratylenchus Filipjev, 1934 and Tylenchus Bastian, 1865 occurring in 90 and 82% of the fields sampled respectively. Least frequently found genera were Hemicycliophora de Man, 1921 and Longidorus Micoletzky, 1922 occurring in 6 and 4% of the fields respectively.

Of the 39 randomly selected strawberry fields, 15 showed excellent growth. In 2 randomly selected fields the entire crop was diseased, and the remaining 22 fields were patchy. Of the 10 fields known to be diseased, 4 were patchy and the additional 4 were uniformly affected by root-rot disease. In the remaining 2 fields numerous plants were affected by "Green Petal" virus disease, but the fields did not appear patchy.

Nematodes proven to parasitize seed plants were found in all but four samples and those

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[1] Contribution No. 1105 from the Research Station, Canada Department of Agriculture, Kentville, Nova Scotia.

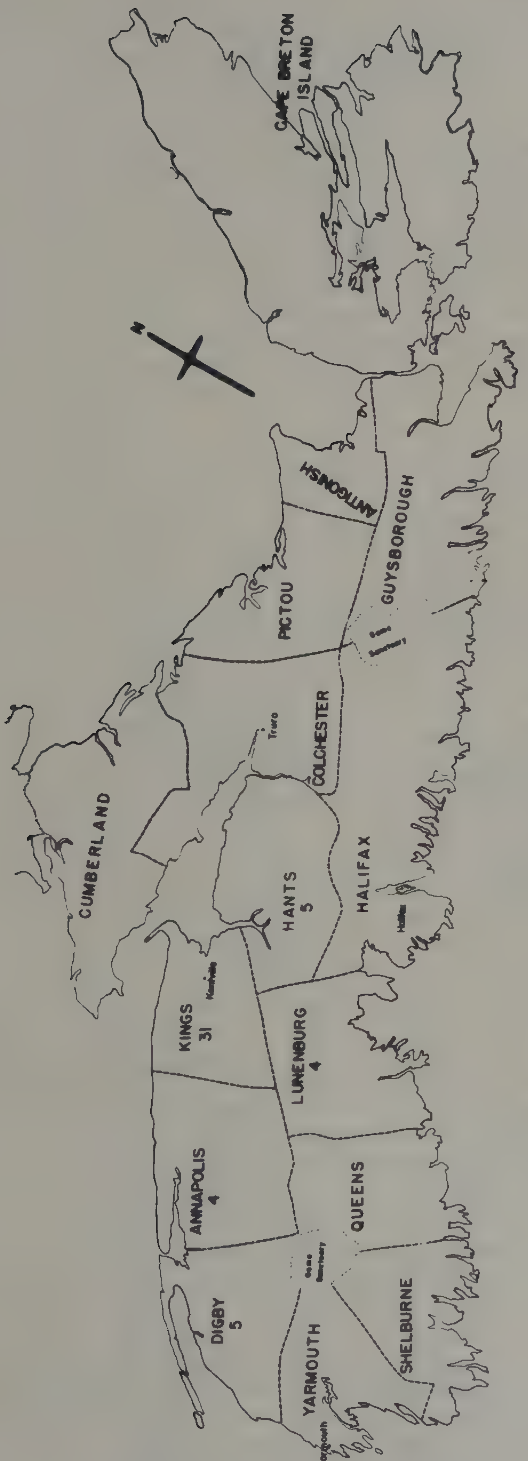

FIGURE 1. Map of Nova Scotia showing areas and number of fields sampled.

four were collected from contiguous plots within a 2-acre site. The strawberry plants in all these plots exhibited excellent growth. However, samples from the other 11 fields in which the strawberry plants appeared healthy did reveal the presence of proven plant parasites in varying numbers (Table 2).

Both samples from the randomly selected fields where the entire crop was diseased revealed the presence of large populations (1000 to 1200) of Pratylenchus penetrans. Each of four samples from the root-rot-affected problem area yielded 1000 to 2000 individuals of Tylenchus spp. It is suspected that Tylenchus spp., the principal stylet-bearing nematodes in those samples, might not have been the initial cause of root-rot because they were found in varying numbers in healthy rhizospheres. Hopper (12) suggested that Tylenchus feeds on deterioration products or fungal organisms causing the root-rot of pine seedlings. Zuckerman (26) listed Tylenchus spp. as nematodes of unknown economic importance.

Of the 26 patchy fields, 15 had larger populations (200 to 1500) of pathogenic nematodes such as Pratylenchus penetrans and Xiphinema americanum in the non-vigorous samples than the corresponding healthy ones (Table 3).

In contrast to Pratylenchus penetrans and Xiphinema americanum, comparable numbers of Pratylenchus crenatus were found in the rhizospheres of both vigorous and non-vigorous plants. Whether this nematode species plays any role in the restriction of strawberry plant growth needs to be determined. Tylenchorhynchus maximus was a frequent associate of both vigorous and non-vigorous plantings. A higher concentration of T. maximus was found in three samples from vigorous plantings than in the corresponding non-vigorous samples. Winslow (25) suspected that Tylenchorhynchus species are "rarely devastating parasites."

Pratylenchus penetrans is considered to be a pathogen of strawberry roots (11, 14), and this was supported by the fact that it occurred in higher concentrations in the rhizospheres of non-vigorous or diseased strawberry plants than in the corresponding vigorous plants. There were two exceptions in which rhizosphere samples from both vigorous and non-vigorous plantings yielded almost equal numbers of P. penetrans. Possibly, factors other than nematodes

Table 1. Frequency at which plant-parasitic nematodes were found in 49 fields.

Nematodes	Frequency (% of samples)
Tylenchus spp.	82
Psilenchus hilarulus	31
Ditylenchus spp.	37
Tylenchorhynchus maximus	49
Neotylenchus spp.	18
Hexatylus spp.	10
Helicotylenchus spp.	22
Rotylenchus robustus	16
Pratylenchus spp.	90
Hemicycliophora sp.	6
Criconemoides lobatum	10
Paratylenchus spp.	14
Aphelenchoides spp.	24
Aphelenchus spp.	36
Xiphinema americanum	37
Longidorus elongatus	4

Table 2. Plant-parasitic nematodes isolated from the rhizospheres of healthy strawberry plants.

Nematodes	No. of fields found (out of 15)	Mean numbers of nematodes per samples (± S. E.)
Pratylenchus crenatus	10	38 (15)
Pratylenchus penetrans	6	86 (39)
Pratylenchus sp.	5	60 (7)
Helicotylenchus spp.	5	18 (5)
Hemicycliophora sp.	1	20 (1)
Tylenchorhynchus maximus	5	125 (48)
Xiphinema americanum	6	12 (4)

Table 3. Plant-parasitic nematodes isolated from vigorous and non-vigorous areas of 26 patchy fields.

Nematodes	Non-vigorous		Vigorous	
	no. of fields found	mean no. found (± S. E.)	no. of fields found	mean no. found (± S. E.)
Tylenchorhynchus maximus	14	42 (14)	10	77 (23)
Helicotylenchus spp.	6	14 (4)	6	9 (3)
Rotylenchus robustus	4	8 (.1)	3	7 (1)
Pratylenchus crenatus	20	50 (17)	19	37 (8)
Pratylenchus penetrans	18	315 (49)	20	92 (28)
Pratylenchus sp.	12	68 (27)	10	33 (7)
Hemicycliophora sp.	2	26 (14)	2	6 (1)
Criconemoides lobatum	3	3 (1)	3	3 (1)
Paratylenchus spp.	6	25 (10)	6	25 (10)
Xiphinema americanum	9	332 (158)	11	41 (15)

caused the differences in vigor of plants in these fields. However, evidence that large populations of P. penetrans might be a factor causing low vigor in strawberry plants came from fields in Bear River, Digby County. Strawberry crops had been grown on these fields over 10 successive seasons. Although many plants showed symptoms of "June Yellows," the remainder lacked vigor as evidenced by small leaves, shortened petioles and inability to produce runners. The root system of 10 randomly withdrawn plants was found to be heavily lesioned and blackened.

Large populations of Xiphinema americanum were found sporadically in four scattered fields and when found were associated with severely stunted and chlorosed strawberry plants.

The survey findings revealed the prevalence of plant-parasitic nematodes of major economic importance, for example, Pratylenchus penetrans reported on tomato (17), potatoes (19, 20), tobacco (18), legumes (13), peaches (16), apples (22), and cherries (15); Xiphinema americanum on laurel oak (5), forest trees and small fruit crops (21, 24); and other parasitic nematodes listed in Table 3. Their significance in crop production in this province needs to be determined. The knowledge of distribution pattern of parasitic nematodes would be of use to extension workers and all engaged in crop production.

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ANTHRACNOSE AND POWDERY MILDEW ON STRAWBERRY PLANTS IN LOUISIANA

N. L. Horn and R. G. Carver[1]

ANTHRACNOSE

An unusually large number of plants died in the field plots at the Louisiana State University Farm, Baton Rouge, in May 1962 from anthracnose caused by the fungus Colletotrichum fragariae A. N. Brooks. When the disease was first reported from Louisiana, in 1960 (2), the authors were more concerned with losses in summer plant beds, and pointed out that the fungus caused a crown rot. In 1962 the organism killed numerous plants in the fruit production stage in the field. This was an unusual occurrence and presented a very striking picture of the effect of the disease (Fig. 1). Counts made on May 25 in five experimental field plots showed that 17% of the 298 Dabreak plants examined were wilted or dead, and 42% of the 278 Headliner plants examined were wilted or dead. Although the data might indicate that Dabreak is more resistant than Headliner, this is not believed to be the case. Because Headliner and Dabreak comprise 90% of the commercial plantings, it is important to note the potential seriousness of the disease in view of the fact that it was prevalent as early as May. A disease of this nature, introduced at this stage in the cycle of plant production in Louisiana, could approach disaster, for the grower depends on healthy field plants grown during the summer for the next season's plants. The disease has been sporadic over the years and observed to occur only during the hot months of June through October.

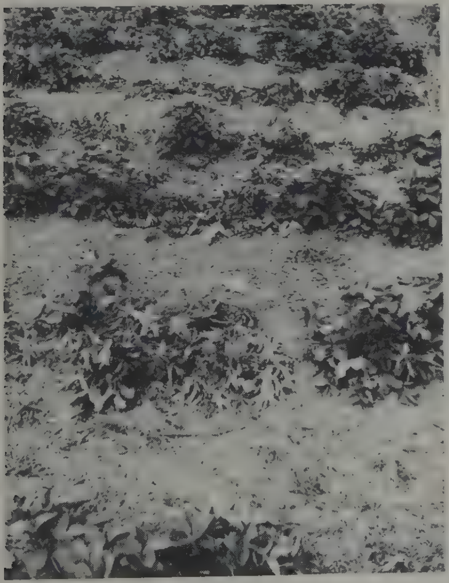

FIGURE 1. Crown rot caused by C. fragariae. Most of the plants here are dead or wilted.

The disease was characterized by a sudden wilt and a crown rot. The fungus attacked the plants at the soil line and grew into and destroyed the vascular system of the stems. No evidence of a root rot or internal discoloration of the roots was present. The infection progressed in lateral and vertical directions, enveloping the entire crown and stem and caused infected plants to wilt and die (Fig. 2).

Cross sections of stems were observed from the time plants started to wilt until they were completely rotted. Tissue from wilted plants was characterized by a light-brown to reddish-brown color. After most of the crown rotted, however, the color of the stems changed to dark brown. Other fungi are known to attack strawberry crowns in Louisiana, but none are known to cause the red color in the stems described here.

Brooks (1) described C. fragariae on strawberry stolons in Florida. In Louisiana the disease was primarily a crown rot.

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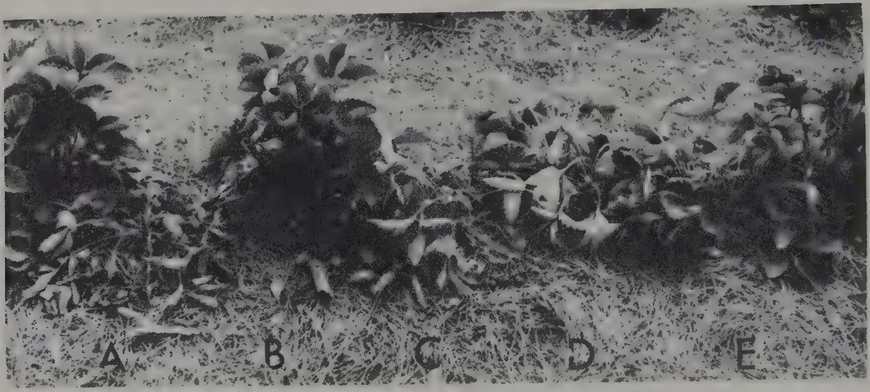

FIGURE 2. Crown rot caused by *C. fragariae*. A -- Dead. B -- Not wilted. C -- Dead. D -- Wilted several days. E -- Wilted 1 day.

Seven hundred isolations were made from infected field plants. *C. fragariae* was consistently found associated with the diseased areas. Plants were artificially infected in the greenhouse by planting healthy plants in soil infested with the fungus which had been grown on a sterilized oat medium. At temperatures above 80°F the organism was found to be pathogenic to the Klonmore, Headliner, Marion Bell and Konvoy varieties.

POWDERY MILDEW

During May 1962, powdery mildew was found on field- and greenhouse-grown Headliner strawberry plants at the Louisiana State University Farm, Baton Rouge. It could not be determined if the causal organism was *Sphaerotheca humuli* (DC.) Burr. (*S. macularis*) because no cleistothecia were found. However, the leaves were covered with the fungus typical of powdery mildew growth from which many conidia of the Oidium stage were found. Since *S. humuli* has been reported from the neighboring States of Arkansas, Alabama, Oklahoma and Texas (3), it is likely that the powdery mildew fungus found in Louisiana may be *S. humuli*. The source of infection was unknown; however, the fungus could have been imported with strawberry plants that were used in a breeding program at the University Farm.

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IN VITRO EFFECTS OF SEVENTEEN CHEMICAL AGENTS ON CYTOSPORA CINCTA[1]

A. E. Harvey and A. W. Helton[2]

Summary

Seventeen compounds were evaluated for in vitro activity against a strain of Cytospora cincta Fr.

Five cycloheximide derivatives, four Omadine derivatives, oxyquinoline citrate, 8-hydroxyquinoline benzoate, Gerox and Mycostatin were highly effective. These compounds will be evaluated for translocation characteristics in tree systems.

INTRODUCTION

Cytospora cankers have been observed on all woody portions of aboveground parts of many kinds of host trees, girdling and killing the terminal portions of the infected branches. Dying terminals "flag" by means of drooping and/or discolored foliage which is reminiscent of fire-blighted twigs of pear trees.

The canker proper is deep-seated and resistant to treatment with surface-contact chemicals (2, 3, 4). Consequently, present control measures emphasize removal and destruction of infected wood (6). Trees infected in the scaffold branches or trunks cannot be saved in this fashion.

This has led to a search for fungicidal compounds which can be translocated efficiently in the conductive systems of diseased trees, thereby affording therapeutic control. Such investigations have been underway in Idaho for several years (1, 2, 3, 4, 5). Potency of new candidate compounds is determined by in vitro evaluation, as herein reported.

MATERIALS AND METHODS

Selection of candidate compounds (Table 1) was based on reports of highly fungicidal and/or systemic properties.

All chemicals were incorporated into Difco malt-extract agar medium containing 12.75 g maltose, 2.75 g dextrin, 2.35 g glycerol, 0.78 g peptone and 15.00 g agar per liter (1).

Each compound was dissolved in sterile distilled water at a concentration of 400 ppm. This solution was added to an equal volume of sterile, double-strength medium to yield a normal-strength medium containing the chemical at 200 ppm. Half of the 200 ppm preparation then was poured into Petri plates and allowed to solidify. The other half was diluted with an equal volume of normal-strength medium, yielding a 100 ppm concentration, half of which was placed in plates and the remainder again diluted with an equal volume of normal-strength medium to yield 50 ppm preparation. This procedure was repeated until the desired 1.57 ppm minimum concentration was reached.

Each plate was inoculated with a 5-mm agar disk containing actively growing mycelium from a virulent Cytospora culture. The disks were inverted in the center of the plates, and all plates were incubated at 27° C. The indicator organism (Cy-16) was isolated from Italian prune in 1954 and identified as a strain of Cytospora cincta Fr. (5).

Effects of the compounds were measured by comparing colony size in the test plates with colony size in the control plates. Readings were taken when the control colonies had reached the edges of the plates, which was approximately 10 days after inoculation. This was done by measuring the diameter of each colony in centimeters, in two directions at right angles to each other, and averaging the two measurements. All four replicates then were averaged, divided by the control average, and multiplied by 100 to obtain percent-of-control figures.

The original inoculation disks were removed from the plates where no growth occurred, placed in plates containing only sterile malt-extract agar and incubated. If growth resulted, the effect of the compound was considered to be fungistatic at that concentration; if no growth occurred, it was fungitoxic.

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[1] Approved by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 550. Gratis samples courtesy of Olin Mathieson Chemical Corp., The Upjohn Company and Midvale Chemical Company.

[2] Respectively, Research Fellow, Department of Plant Pathology, University of Idaho and Associate Plant Pathologist, Idaho Agricultural Experiment Station.

Table 1. Chemical agents tested for activity against *Cytospora* isolate Cy-16 on malt-extract agar.

Active compound	Trade or code name
Cycloheximide (Beta-(2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl)-glutarimide)	Actidione
Cycloheximide thiosemicarbazone	-
Cycloheximide carbazone	-
Cycloheximide oxime	-
Cycloheximide methylhydrazone	-
Cycloheximide acetoacetate	-
Cycloheximide acetate	-
Manganese 2-pyridinethiol, 1-oxide	Mn-Omadine
Sodium 2-pyridinethiol, 1-oxide	Omadine-Na salt
1-oxide-2-pyridylthiomethyl propyl ether	Propoxymethyl-Omadine
S-1(1-oxido-2 pyridyl) isothiuronium chloride	Thiourea-Omadine
Oxyquinoline citrate (8-hydroxyquinoline citrate)	Citroxin
Streptomycin sulfate 25.3%, oxyquinoline sulfate 31.6%, and inert 43.1%	Gerox
Iron (chelated)	Sequestrene Iron Chelate
8-azaquanine	-
8-hydroxyquinoline benzoate	-
-	Mycostatin

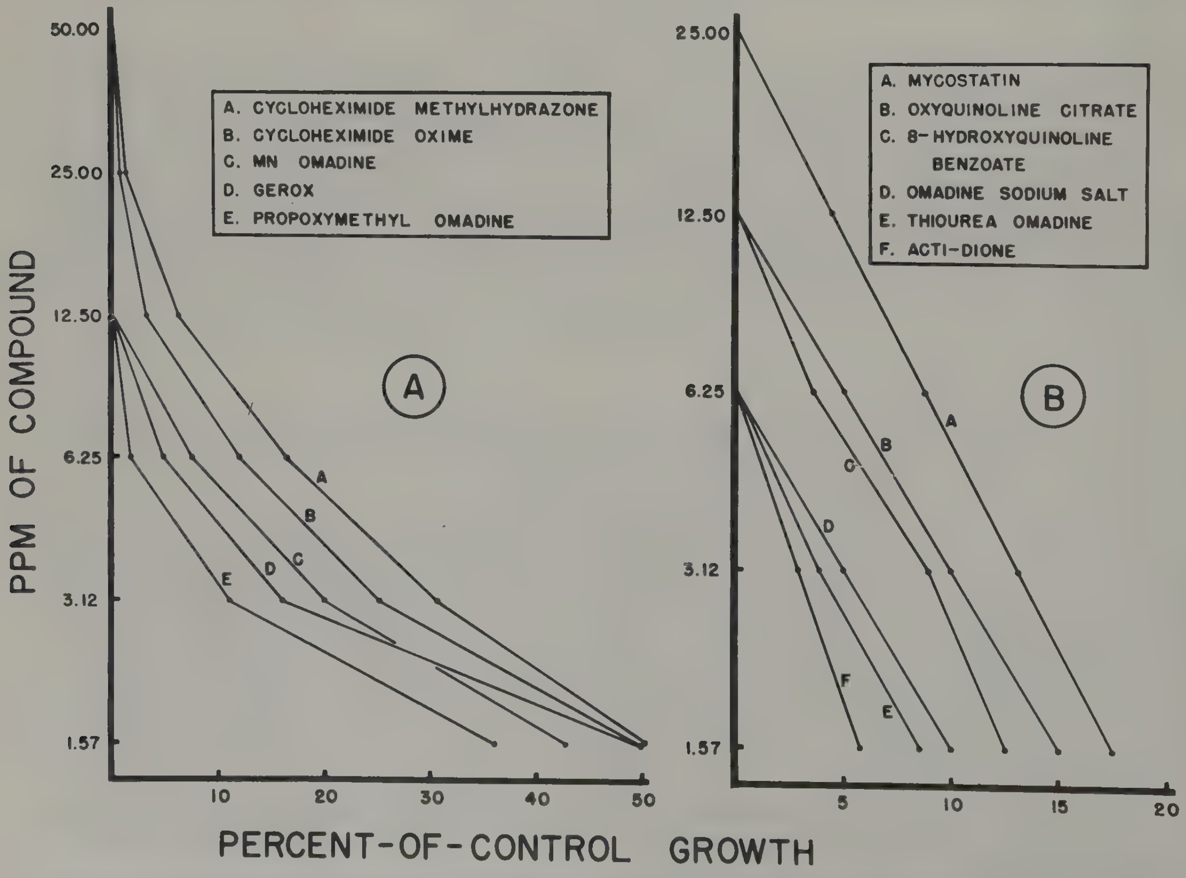

FIGURE 1. Effect of 11 test compounds on *in vitro* growth of *Cytospora* isolate Cy-16 when various concentrations were incorporated into a malt-extract agar medium, inoculated, and incubated at 27° C for 10 days.

Dimethyl sulfoxide was used as a solvent for the relatively insoluble cycloheximide derivatives in preparation of sterile-water solutions. A concentration of 850 ppm was satisfactory, that is, effective as a solvent and non-toxic to the fungus.

RESULTS

Sequestrene iron chelate and 8-azaquanine did not inhibit the growth rate of the test fungus. The growth habit, however, was altered by 8-azaquanine. The periphery of the colony was relatively smooth in the control plates. The presence of 8-azaquanine, at all concentrations, changed the normally smooth margin to an erratic margin with projecting, ropy masses of hyphae.

Cycloheximide acetate and cycloheximide acetoacetate yielded almost identical results. They were lethal at 400 ppm and 20% inhibitory at 50 ppm. The latter was somewhat more inhibitory than the former at 200 ppm. Neither compound caused detectable growth suppression at 25 ppm.

Cycloheximide methylhydrazone, cycloheximide oxime, Mn-Omadine, propoxymethyl-Omadine and Gerox yielded inhibition curves which indicated that the fungicidal activity of these compounds decreased at an increasing rate as the concentration was decreased below the toxic level (Fig. 1A). Cycloheximide methylhydrazone and cycloheximide oxime were lethal at 50 ppm and inhibitory at 1. 57 ppm, the lowest concentration tested. Mn-Omadine, propoxymethyl-Omadine and Gerox were lethal at 12. 5 ppm and inhibitory at 1. 57 ppm.

Mycostatin, oxyquinoline citrate, 8-hydroxyquinoline benzoate, Actidione, thiourea-Omadine and Omadine-Na salt yielded inhibition curves indicating that the activity of each compound decreased at an approximately constant rate with reduction in concentration below the toxic level (Fig. 1B). Mycostatin was lethal at 25 ppm and inhibitory at 1. 57 ppm. Oxyquinoline citrate and 8-hydroxyquinoline benzoate were lethal at 12. 5 ppm and inhibitory at 1. 57 ppm. Actidione, thiourea-Omadine and Omadine-Na salt were lethal at 6. 25 ppm and inhibitory at 1. 57 ppm.

The greatest activity was exhibited by cycloheximide semicarbazone and cycloheximide thiosemicarbazone. These compounds were lethal at 3. 12 ppm and highly inhibitory at 1. 57 ppm. Since only one concentration was tested at which these materials were not lethal, inhibition curves could not be constructed and they are not represented in Figure 1.

Changes in pH with dilution were minor in all cases. By means of aliquot testing with a Beckman meter, hydrogen-ion concentrations were checked for each material after it was incorporated into the agar medium. The pH tests were repeated 5 days after inoculation of the plates, and again after 10 days, that is, on completion of the tests. The pH level generally decreased with time, increased in some cases, but never changed more than 0. 75 from one testing to another. The lowest pH level found was 5. 0 and the highest was 6. 5.

DISCUSSION AND CONCLUSIONS

Although all cycloheximide derivatives were highly fungitoxic, cycloheximide acetate and cycloheximide acetoacetate were the least effective. This suggests that the acetate grouping is not as toxic to Cytospora as others represented among the cycloheximide derivatives.

The explanation for one group of the compounds decreasing in activity at an increasing rate with reduction in concentration, and another decreasing at a nearly constant rate, is not known (compare Figs. 1A, 1B). These activity patterns suggest, however, that the compounds included within the former group may not be so lastingly effective as therapeutants in the diluting systems of fruit trees as the compounds of the latter group. Since pH changes with dilution were minor, and since the pH levels were well within the pH range of good Cytospora growth (7), the explanation cannot lie in pH effects.

Results obtained with cycloheximide and with oxyquinoline citrate are in agreement with those reported previously (1, 5).

It is especially interesting that two Omadine derivatives were in one group and two in the other. This may yield structural clues that will be of value in future syntheses of candidate compounds.

With the exception of Sequestrene iron chelate, 8-azaquanine, cycloheximide acetoacetate and cycloheximide acetate, all compounds tested were highly active as fungitoxicants. Because of this activity, the remaining 13 materials will be evaluated for therapeutic activity in subsequent investigations.

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ALFALFA SEEDLING LOSSES AT VARYING TIMES AFTER PLANTING[1]

A. F. Schmitthenner and J. L. Parsons[2]

Summary

Poor emergence was not a factor in stand establishment of alfalfa in both field and greenhouse tests. The incidence of postemergence seedling root rot was high in several soils in which establishment of alfalfa had been unsuccessful. The greatest seedling kill occurred during the early summer of the seeding year. First-year overwintering loss was negligible.

INTRODUCTION

Considerable difficulty has been experienced in some areas of Ohio in establishment of alfalfa stands. Ten pounds of seed are generally broadcast in the spring in fall-planted wheat, or band seeded with spring-planted oats. In areas where there has been difficulty in establishing alfalfa, few plants survive until the spring of the year following seeding. Various species of Pythium and Fusarium, and Rhizoctonia solani Kuehn, have been associated with damping-off of alfalfa seedlings (2, 6, 10). Other fungi such as Phytophthora cryptogea Pethy. & Laff. (3, 5), or the many crown rot organisms (4, 8, 10) are capable of damaging older plants. This study was made to gain information on the relationship of pre-emergence and postemergence seedling loss, loss of older plants during the first summer, and loss over the first winter, to alfalfa seeding failures. The fungi associated with alfalfa seedling kill will be reported elsewhere.

MATERIALS AND METHODS

Pre-emergence seedling loss was evaluated by comparing untreated Atlantic alfalfa seed with seed of the same lot treated with thiram (Arasan 50) at the rate of 1 g/240 g seed. In greenhouse tests four replications/treatment, each consisting of 100 seeds/15 x 15 x 30-cm box of field soil, were planted in 28-cm furrows 2 cm deep in water-saturated soil and covered with dry soil. The field plantings were band-seeded in two locations at the Experiment Station Farm, Wooster, Ohio, without a companion crop in May in three and four replications of 7 x 50-foot plots of 8-inch rows for tests A and B, respectively. Two to 3 weeks after planting all of the seedlings in the greenhouse tests were counted and dug up for examination of the roots. Two 8-inch sections of each row (16 subsamples/plot) were counted and dug for root examination in field test A, while one 8-inch section from each row (8 subsamples/plot) was sampled in field test B. All tests were in Wooster silt loam.

Several field studies were made using a "List Quadrat" consisting of 1-foot sections of row which were labeled with a permanent, numbered marker so that each quadrat could be located and counted several times during the year following seeding. Three tests, one each at Wooster, the Northwest Substation in Wood County, and the Southeast Substation in Meigs County, Ohio, were made in 1956 and will be referred to as quadrat tests W_1, N, and S, respectively. Test W_1 consisted of 24 quadrats randomly distributed in twelve 5 x 20-foot plots, each containing six rows of Atlantic alfalfa. This test was planted in early June, and the quadrats counted in late June, October, and April of the following year. Test N consisted of 120 quadrats in an alfalfa variety test planted in 6 x 20-foot plots of five rows each in early June. Counts of the quadrats of this test were also made in late June, October, and April of the following year. As no differences among varieties were noted, the data were subjected to an analysis of variance considering the varieties as subsamples. Test S consisted of 120 quadrats randomly distributed in Buffalo alfalfa broadcast on fall-planted wheat in March. Counts were made in May, June, July, August, October, and in the following spring.

A more precise study was made at the same time at Wooster, Ohio, and is designated W_2. In early March Ranger alfalfa was broadcast in fall-planted wheat in March. Fifty quadrats were set up and counts were made in April, June, August, and October. At the first count the quadrats were grouped into one of four categories, depending on the number of seedlings, so that intraspecies competition could be evaluated.

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[1] Approved as Journal Article No. 45-62 of the Ohio Agricultural Experiment Station, Wooster.

[2] Associate Professor, Department of Botany and Plant Pathology, and Associate Professor, Department of Agronomy, respectively, Ohio Agricultural Experiment Station, Wooster and The Ohio State University, Columbus.

RESULTS AND DISCUSSION

There were no differences in emergence between treated and untreated seed in either greenhouse or field tests. The effect of seed treatment on emergence in the field tests is summarized in Table 1. The incidence of seedling root rot was 75 and 79% in the two greenhouse tests, respectively, and seed treatment did not control seedling root rot. In one field test there was less seedling root rot in the seed treatment plots (Table 1). The seedling root rot observed was similar to that described by other workers as being caused by Pythium (2, 6). Similar results have been obtained in subsequent tests using a number of different soils. In the greenhouse, seedlings often recover with the proliferation of secondary roots. Under field conditions many seedlings disappeared by October (Table 1).

Table 1. Effect of seed treatment on disease and stand of Atlantic alfalfa in two field trials at the Experiment Station Farm, Wooster, Ohio.

Field	No seed treatment			Arasan seed treatment		
	3 weeks		1 year	3 weeks		1 year
test[a]	stand[b]	% disease[c]	no surviving[d]	stand[b]	% disease[c]	no surviving[d]
A	15.7	--	2.4	14.0	--	2.3
B	20.3	64	2.9	23.3	39	2.9

[a] Three and four replications of 7 x 50-foot plots of 8-inch rows for tests A and B, respectively. Two and one 8-inch sections of each row were dug for root examination in tests A and B, respectively.

[b] Mean number of plants per foot of row 3 weeks after planting.

[c] Mean % of plants with seedling root rot.

[d] Mean number of plants per foot of row 1 year after planting.

The quadrat studies were made to determine more accurately when seedling loss occurred. The data from tests W_1, N, and S are indicated in Figure 1, depicting the S values for June, October, and the following spring, only, to correspond to the W_1 and N data. At the N location, the number of seedlings surviving in October was significantly less than the number in June, but not significantly different from the number the following spring. The data from the W_2 study are summarized in Figure 2. The greatest seedling loss occurred during early summer and in the best initial stands.

In this study the greatest stand loss occurred during the summer of the seeding year before such winter problems as heaving, such spring problems as Sclerotinia crown rot or Phoma shoot kill and black stem, or such older plant problems as crown bud rot (8) or bacterial wilt. Most papers dealing with alfalfa seedling kill emphasize pre-emergence damping-off and its control, but in the tests reported in this study postemergence seedling root rot was of greater importance. Several recent papers on seed treatment of alfalfa (1, 9, 11, 12) reported that seed treatment had little effect on stands under field conditions. Halpin and Hanson (7) reported that alfalfa becomes resistant to a number of Pythium species within 3 days after germination. In the tests reported above, rotting of the tap root often occurred 2 to 3 inches below the soil surface, indicating that infection occurred in plants older than 3 days. This agrees with observations of Buchholtz (2). It is also possible that other pathogens are involved in alfalfa seedling root rot which produce symptoms indistinguishable from Pythium.

In the present study the relationship between seedling root rot and loss of seedlings was not clear. Many of the seedlings with root rot appeared to be recovering with the initiation of secondary roots, yet there was considerable seedling loss during the summer. Perhaps disease-weakened plants were unable to survive in competition with associated wheat or weeds, or some other disease problem affected the seedlings later in the summer. Seedling root rot was rare in late July or August seedings, and in seedings made in field soil collected during this period by the authors. It was not known whether the pathogens were less abundant at this time of the year, or whether the environment was unfavorable for initiation of root rot. Further studies should be concentrated on the pathogens involved and the factors affecting the development and severity of root rot of alfalfa seedlings.

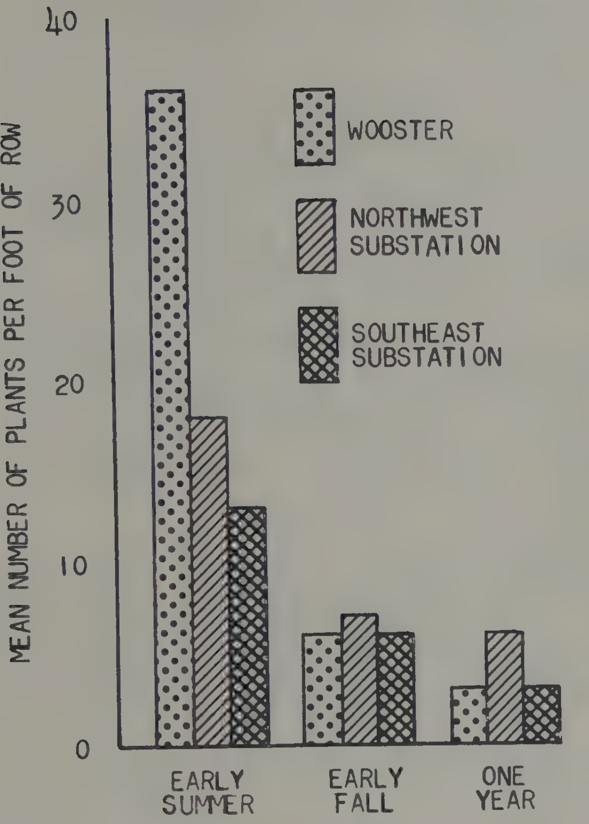
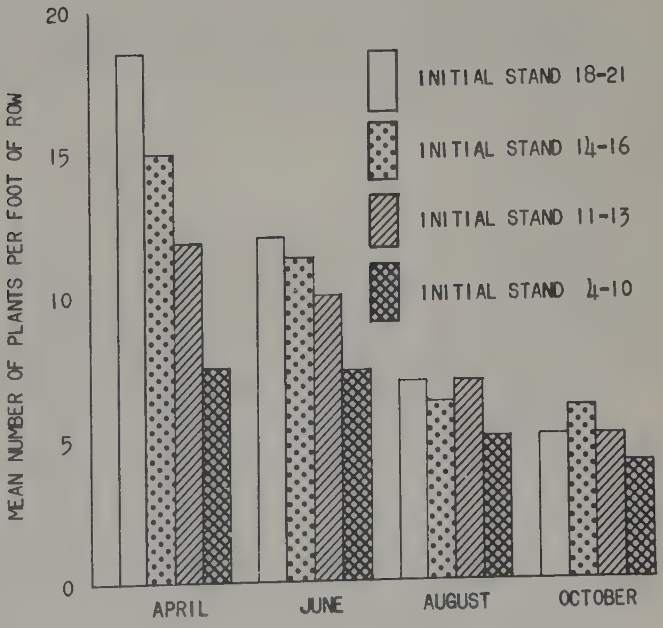


FIGURE 1. Alfalfa seedling stands in early summer, early fall, and 1 year after seeding at three locations. The Wooster test (W_1) consisted of 24 quadrats randomly distributed in twelve 5 x 20-foot plots of Atlantic alfalfa planted in early June; the Northwest Substation test consisted of 120 quadrats in an alfalfa variety test planted in 6 x 20-foot plots in early June; and the Southeast Substation test (S) consisted of 120 quadrats randomly distributed in Buffalo alfalfa broadcast on fall-planted wheat in March.

FIGURE 2. Atlantic alfalfa populations 1, 3, 5, and 7 months after broadcasting seed in wheat in March at the Wooster Experiment Farm. Fifty quadrats were separated into four different density groups based on the number of seedlings present at the first count in April.



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NEW SOIL FUNGICIDES FOR POTATO SCAB AND RHIZOCTONIA CONTROL

R. C. Cetas[1] and E. D. Jones[2]

Abstract

Preplanting band applications of CP-30249 and of DAC-649 accounted for a significant reduction in the severity of common scab of potatoes. CP-30249 was more effective when incorporated with the soil to a depth of 5 to 6 inches than of 2 to 3 inches. Depth of incorporation did not affect the efficacy of DAC-649. DAC-649 was ineffective when left on the soil surface for 4 to 5 hours before planting. Foliar applications of phosphamidon decreased the incidence of scab slightly.

Preplanting band applications of 40 and 60 pounds active DAC-649 per acre delayed emergence of Kennebec potato plants and delayed the development of the symptoms of early maturity wilt.

Preplanting broadcast application of DAC-649 in the spring accounted for a significant reduction in the severity of Rhizoctonia stem canker. It failed, however, to control the sclerotial phase of the disease. Fall applications of DAC-649, PCNB, and Vapam were ineffective against both phases of the Rhizoctonia disease.

INTRODUCTION

Common scab, caused by Streptomyces scabies (Thaxt.) Waks. & Henrici, is one of the major diseases of potato (Solanum tuberosum). The maintenance of the soil reaction between pH 4.8 and 5.2 and the use of resistant varieties have been the chief controls. Scab has been observed in soils with a pH of 4.3. Consequently, growers have been reluctant to lime soils with a pH below 4.8. With an effective chemical soil treatment, growers could practice liming which would reduce the detrimental effects of high acidity and would allow a greater variety of crops to be grown in the rotation.

A number of soil fungicides have been evaluated by various workers. Of these, pentachloronitrobenzene (PCNB) and urea formaldehyde concentrate-85 (UF-85 or Uracide) have received the most attention (1, 2, 4, 5, 6, 7, 8, and others). PCNB and Uracide have not been recommended to growers in New York, either because the treatment was too expensive or because the fungicide did not control scab satisfactorily (2, 7).

In a greenhouse test, thorough incorporation of 40 and 80 pounds of 3,3,4,4,-tetrachlorotetrahydrothiopene-1-1-dioxide (DAC-649) per acre resulted in exceptional control of scab in Sassafras silt loam soil naturally infested with S. scabies and with a pH of about 6.0 before treatment. In 1961, field experiments were conducted in upstate New York and on Long Island to evaluate DAC-649 and other soil fungicides further for the control of common scab, Rhizoctonia (caused by Rhizoctonia solani Kuehn) and other diseases of potatoes caused by soil-inhabiting pathogens.

MATERIALS AND METHODS

Long Island Experiments: Four experiments, three against scab and one against early maturity wilt (9) were conducted on Sassafras silt loam soils. One scab experiment was conducted in two replications of 13 randomized permanent pH plots that ranged from pH 4.3 to 8.0. The desired pH has been maintained by annual applications of lime or sulfur. Each plot measured 16.5 x 38 feet. The plots in one replication were given broadcast treatments of 40 pounds active DAC-649[3] (1:9 mixture of 50% wettable powder and dry builder's sand) in the fall of 1960. The plots in the other replications were not treated. The fungicide was incorporated immediately with a disk harrow and a rye cover crop was sown the same day. The plots were planted with the Katahdin variety in the spring of 1961. At harvest, yields were taken from 30 feet of row of each plot.

The randomized block with three replicates was used in the other two scab tests. Each plot consisted of two 45-foot rows of Green Mountain potatoes. Fifteen days after harvest the soil pH ranged from 5.5 to 5.8. The soil fungicides were applied in an 18-inch band centered on the row with a gravity-flow granular applicator (Fig. 1) (3). In one test, DAC-649 (1:9 mixture of 50% wettable powder and dry sand) and 2-chloro-3-(tolysulfonyl)-propionitrile (CP-30249, 5%

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[3] Supplied by Diamond Alkali Company.

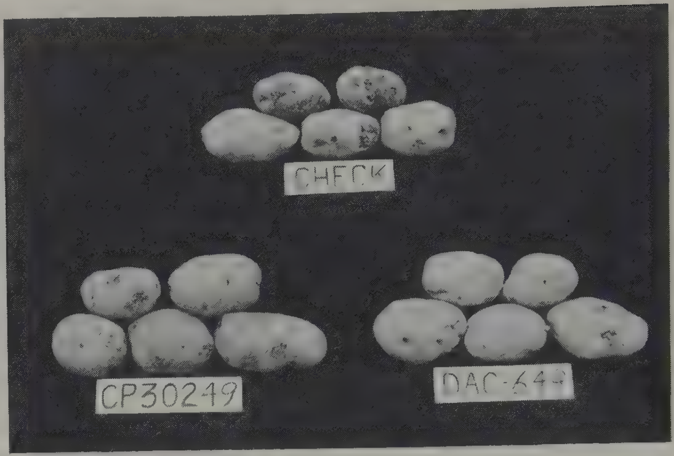

FIGURE 1. (Left) Gravity-flow granular applicator used to make preplanting band applications of soil fungicides. (Photograph supplied by W. W. Gunkel, Department of Agricultural Engineering, Cornell U.)

FIGURE 2. (Above) Green Mountain potato tubers grown in soils given preplanting band applications of 60 pounds active of CP-30249 and of DAC-649. Fungicides thoroughly mixed with the soil to a depth of 5 to 6 inches with a rotovator immediately after application and about 5 hours before planting on May 1, 1961. (Photographed by H. S. Cunningham)

granule[4]) were applied at the rate of 60 pounds active per acre on May 1. One row of each plot was rotovated to a depth of 2 to 3 inches immediately after application and the other row to a depth of 5 to 6 inches. The plots were planted about 4 hours later. Between June 23 and September 6, 12 foliar applications, one each week, of 1-chloro-1-diethylcarbamoyl-1-propen-2-yl dimethyl phosphate (phosphamidon, 4 pounds/gallon[5]) were applied to two rows in each replicate. Phosphamidon was applied at the rate of 1 pint per 100 gallons per acre per application with a tractor-mounted high pressure sprayer. In the other scab trial the 1:9 50% DAC-649-sand mixture was applied at the rate of 20, 40, and 60 pounds active per acre on June 16. One row of each plot was rotovated immediately to a depth of 5 to 6 inches. The fungicide remained on the soil surface of the other row for about 5 hours and was mixed with the soil as the planter opened and closed the seed furrows. At harvest, yields were taken from 45 feet of each row in both experiments.

In all three scab tests, tubers were graded over a 2-inch screen. Forty tubers over 2 inches in diameter were washed and classified for type of scab lesion and surface area covered (Table 1). A numerical rating[6] was assigned to each combination of lesion type and surface area covered class (Table 2). The numerical ratings were arbitrarily weighted values based on the degree of scab severity (surface area covered and lesion type). To determine the numerical ratings, weighted values of 1, 2, 4, 6, 10 and 14 were established for surface area classes T, 1, 2, 3, 4, and 5 respectively. These values in turn were multiplied by the common factors of 1, 2, 6 and 10 to determine the numerical ratings for tubers with lesion types 1, 2, 3 and 4 respectively. The scab index for each plot was the sum of individual tuber ratings divided by the total number of tubers examined. The lowest possible index was 0 and the highest 140. A plot or treatment index of 8 or less could be considered as commercial control.

The fourth experiment, exploratory in nature, was conducted in a field in which early maturity wilt was a problem. Verticillium albo-atrum Reinke & Berth. (microsclerotial form), Fusarium spp. and Pratylenchus sp. (meadow nematode) have been associated with the disease in this field. The 1:9 50% DAC-649-sand mixture was applied at the rate of 20, 40 and 60 pounds active per acre in 18-inch bands centered on the row on May 1. Two 100-foot rows were given each dosage. The 20- and 40-pound dosages were replicated twice. The 60-pound dosage was not replicated. Immediately following application, one row of each plot was rotovated 5 to 6 inches deep. The fungicide was mixed immediately with the soil by the potato planter in the other row. Tubers were harvested from 25 feet of each row on October 9. Forty tubers over 1 7/8 inches in diameter were examined for scab, brown-eye and stem-end browning. The latter two defects have been associated with Verticillium wilt by many workers.

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[4] Supplied by Monsanto Chemical Company.

[5] Supplied by California Chemical Company.

[6] The numerical ratings were determined in cooperation with Dr. L. C. Peterson, Department of Plant Pathology, Cornell University.

Upstate Experiment: DAC-649 (10% dust[3]) PCNB (10% dust[7]) and sodium n-methyl dithiocarbamate (Vapam[8]) were evaluated for the control of common scab and the stem canker and sclerotial phases of the Rhizoctonia disease. All treatments were applied in the fall except for a spring application of DAC-649. Both PCNB and DAC-649 were broadcasted by hand and incorporated with a disk harrow. The spring application of DAC-649 was made just prior to planting. Vapam was applied with a gravity flow shank applicator and immediately after its application, an inch of water was applied by overhead irrigation. The soil temperature was 55° F at time of application.

The test was conducted with the Katahdin variety on sandy loam soil in Oswego County. A randomized block design with five replications was used. Each plot consisted of six 30-foot rows. Approximately 100 plants, 8 to 10 inches tall, were pulled from rows 2 and 5 of each plot and rated for stem canker. The average rating for all scored stems was used as the stem canker index for each treatment (Table 6). At harvest yields were taken from 20 feet of the center two rows of each plot. Approximately 100 tubers selected at random from each plot were rated for total surface area covered by Rhizoctonia sclerotia (Table 6) and for common scab severity (Tables 1 and 2). The average ratings for all scored tubers within a treatment determined the disease index.

Table 1. Classes used in rating common scab severity (surface area covered and lesion type).

Class	Description
Surface area covered:	
0	none present
T	trace
1	1 - 10%
2	11 - 20%
3	21 - 40%
4	41 - 60%
5	61 - 100%
Lesion type:	
0	none present
1	surface or russet type
2	raised and slightly pitted
3	pitted
4	deep pits

Table 2. Numerical ratings used in determining scab indices.

Classes for surface area covered	Classes for type of scab lesion				
	0	1	2	3	4
0	-	-	-	-	-
T	-	1	2	6	10
1	-	2	4	12	20
2	-	4	8	24	40
3	-	6	12	36	60
4	-	10	20	60	100
5	-	14	28	84	140

$$\text{Scab index} = \frac{\text{Sum of individual tuber ratings}}{\text{Total number of tubers examined}}$$

RESULTS AND DISCUSSION

Long Island Experiments: Foliar sprays of phosphamidon resulted in a slight decrease in the incidence of common scab but the control obtained was unsatisfactory (Table 3). Preplanting band applications of CP-30249 and of DAC-649 resulted in an appreciable reduction in the incidence and severity of scab when the fungicides were incorporated with the soil immediately after application (Fig. 2, Tables 3 and 4). CP-30249 was more effective when mixed with the soil to a depth of 5 to 6 inches than to a depth of 2 to 3 inches. Depth of incorporation did not affect the effectiveness of DAC-649. When incorporated immediately with the soil, preplanting band application of 20 pounds active DAC-649 per acre was as effective as the higher dosages. DAC-649, however, was ineffective when left on the soil surface for 4 to 5 hours before incorporation with the potato planter (Table 4).

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[7] Supplied by Olin Mathieson Chemical Corporation.
[8] Supplied by Stauffer Chemical Company.

Table 3. Yield and incidence and severity of scab of Green Mountain potatoes given 12 weekly sprays of phosphamidon, or grown in soils given preplanting band applications of 60 pounds active of CP-30249 or DAC-649 per acre, 1961.

Treatment	Yield over 2 inches bushels/acre	% of tubers affected with scab	Scab index[a]
None	512	97.9	50.3
Pnosphamidon, 1 pint/acre, 12 weekly applications	543	87.9	47.5
CP-30249 2 - 3 inches deep	473	59.2**	12.4**
5 - 6 inches deep	531	39.2**	4.2**
DAC-649 2 - 3 inches deep	501	33.3**	3.8**
5 - 6 inches deep	506	35.8**	6.5**

** Significant at 1% level when compared with "None."

[a] Lowest possible index was 0, highest possible 140, see Tables 1 and 2.

Table 4. Yield and incidence and severity of scab of Green Mountain potatoes grown in soils given preplanting band applications of DAC-649, 1961.

Pounds active/acre in 18-inch band and method of incorporation	Yield over 2 inches bushels/acre	% of tubers affected with scab	Scab index[a]
None	230	50.4	8.0
20 - planter	285	40.8	5.5
rotovator	220	10.0**	0.8*
40 - planter	236	58.3	8.7
rotovator	210	12.5*	0.5*
60 - planter	236	40.8	4.7
rotovator	194	7.5**	1.2*

* Significant at the 5% level when compared with "None."

**Significant at the 1% level when compared with "None."

[a] See footnote a Table 3.

Table 5. Yield and incidence of scab, brown-eye and stem-end browning of Kennebec tubers grown in soils given preplanting band applications of DAC-649, 1961.

Pounds active/acre in 18-inch band and method of incorporation	Yield over 1 7/8 inches bushels/acre	% of tubers affected with:		
		scab	brown-eye	stem-end browning
None	435	16	13	31
20 - planter	403	5	14	30
rotovator	428	0	8	21
40 - planter	474	0	6	16
rotovator	430	0	5	15
60 - planter	400	0	5	15
rotovator	390	0	10	25

Table 6. The effect of DAC-649, PCNB and Vapam upon the control of *Rhizoctonia solani*.

Soil treatment	Stem canker Index[a]	Sclerotia Index[b]	Yield over 2 inches bushels/acre
None	1.1 (58)[c]	1.0 (77)[d]	494
DAC-649 - 10% dust 400 pounds/acre (brd., fall)	.9 (50)	1.0 (72)	528
DAC-649 - 10% dust 400 pounds/acre (brd., spring)	.4** (31)	1.2 (80)	533
PCNB - 10% dust 500 pounds/acre (brd., fall)	.9 (49)	1.1 (79)	522
Vapam 40 gallons/acre (injected fall)	1.3 (63)	1.3 (90)	520
Vapam 80 gallons/acre (injected fall)	1.0 (49)	1.3 (84)	545

** Significant at the 1% level when compared with "None."

[a] Average rating for all scored stems within a treatment - stems rated according to pictorial classes based on the number and type of cankers.

(0 = No cankers; 1 = few superficial cankers; 2 = many superficial or mixture of superficial and 2 deep cankers; 3 = 3 or more deep cankers; 4 = stem completely girdled).

[b] Average rating for all scored tubers within a treatment - tubers rated according to pictorial classes based on total surface covered by sclerotia.

(0 = no sclerotia; 1 = 0.5% or less; 2 = 1%; 3 = 3%; 4= 5%; 5 = 7% or more).

[c] % stems with cankers.

[d] % tubers with sclerotia.

A few very scabby tubers were found in the plots given the more effective band treatments of CP-30249 and DAC-649. Observations indicated that the very scabby tubers developed either beneath or outside the treated band of soil. The majority of the scabby tubers in these plots were affected with either the russet or raised pustule type of scab. Usually the pustules had not erupted. Consequently, the crop harvested from the plots given the more effective CP-30249 and DAC-649 treatments could have been graded to U.S. No. 1 specifications. In contrast, the crop harvested from the check plots was a total loss. Similar results have been obtained with DAC-649 in Michigan (8).

Broadcast application of 40 pounds active DAC-649 in the fall resulted in a slight increase in yield and decrease in scab incidence: Check - 484 bushels per acre of tubers over a 2-inch screen, scab index of 2.4, 63% of the tubers affected; DAC-649 treated plots - 504 bushels per acre, scab index of 1.2, and 42% of the tubers affected. The differences, however, were not significant at the 5% level. The DAC-649 treatment reduced the stand of the rye cover crop by about 50%.

The band applications of DAC-649 just prior to planting delayed emergence of Kennebec potato plants from 2 to 8 days in the test against early maturity wilt; the higher the dosage the greater was the delay. At each dosage, the delay in emergence was about 2 days longer when the fungicide was incorporated with the soil with the rotovator than with the potato planter during the planting operation. The effect of the delayed emergence was evident until after the plants in the untreated plots filled the rows. Band application of 20 pounds active DAC-649 per acre did not delay the development of the symptoms of early maturity wilt but the use of the higher dosages did. The highest yields occurred in the plots treated with 40 pounds active per acre and incorporated with the potato planter (Table 5). Band application of 40 and 60 pounds active per acre resulted in a decrease in the incidence of scab, brown-eye and stem-end browning.

Upstate Experiment: The spring application of DAC-649 was the only treatment to account for an appreciable reduction (significant at the 1% level) in the number and severity of Rhizoctonia stem cankers (Table 6). Of the plants examined, 69% were free from stem cankers as compared with 42% in the untreated plots. None of the treatments were effective in controlling the sclerotial (black scurf) phase of the disease.

The incidence of scab within the trial was too low to provide a test. An increase in yield ranging from 26 to 51 bushels per acre was noted in all chemically treated plots.

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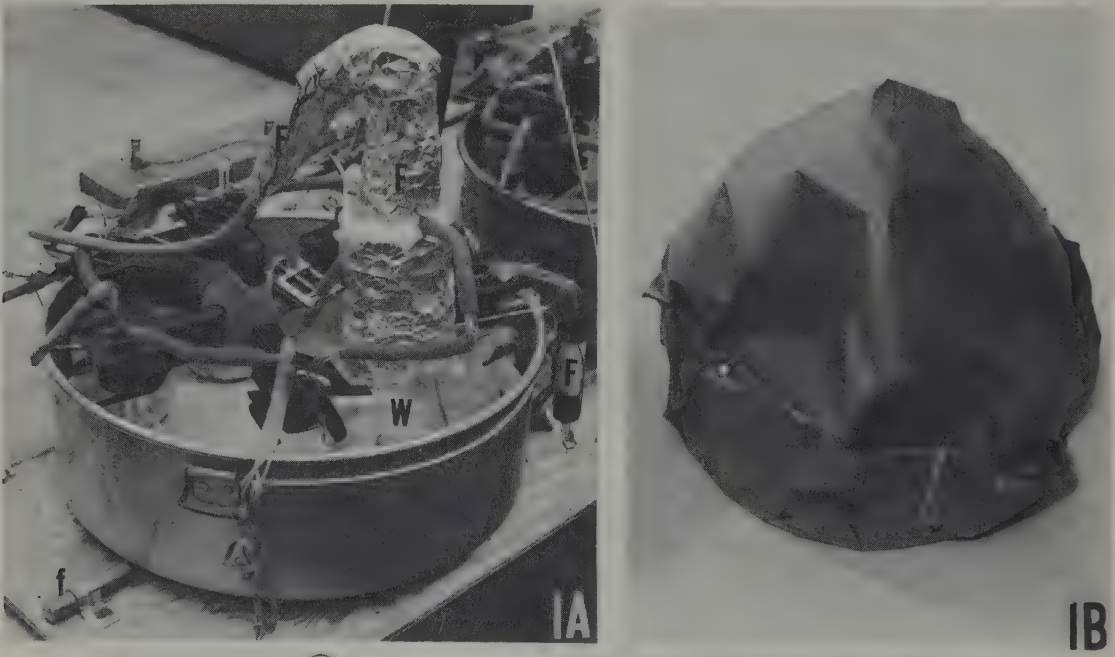
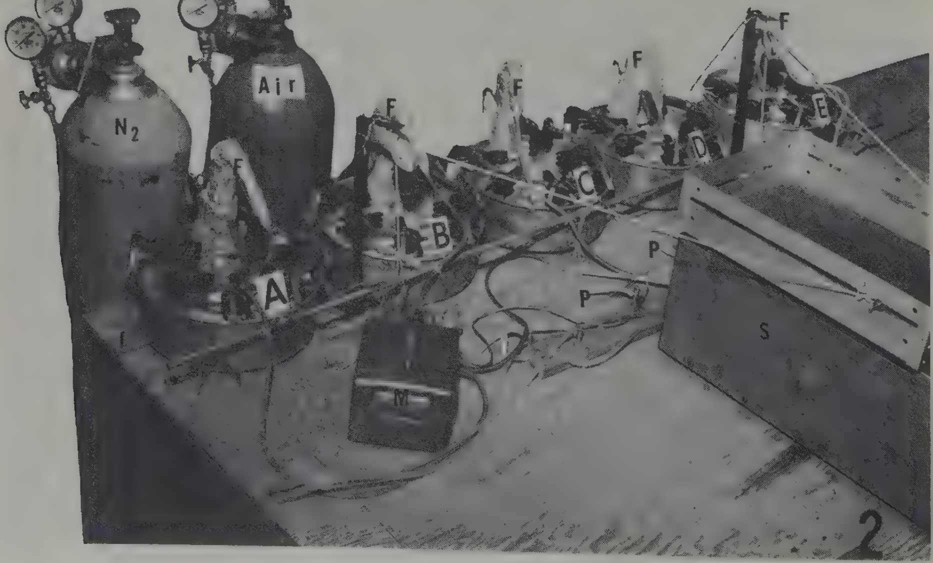
A METHOD FOR DETERMINING THE OXYGEN REQUIREMENT OF FUNGI IN LIQUID MEDIA

L. J. Klotz, L. H. Stolzy, and T. A. DeWolfe

Investigations with Phytophthora spp. that attack citrus roots have suggested that a better understanding of the conditions of moisture and aeration in soils is necessary for defining the nature of environment required for pathogenesis by these and other root-attacking fungi. A method was developed for estimating the oxygen requirements of the pathogens growing in liquid media. The method is illustrated in Figures 1, 2, and 3. Difco potato-broth containing 0.2% yeast extract was used. A small piece of marble ($CaCO_3$) was added to buffer against large changes in H-ion concentration during growth of the fungi. Prior to autoclaving the medium in 125-ml flasks, the top of each flask was covered with aluminum foil and each entire unit of six flasks was carefully enclosed in wrapping paper (Fig. 1B). In these trials 15- and 25-ml quantities of the broth were inoculated with a 5-mm disk of mycelium from a Petri dish culture





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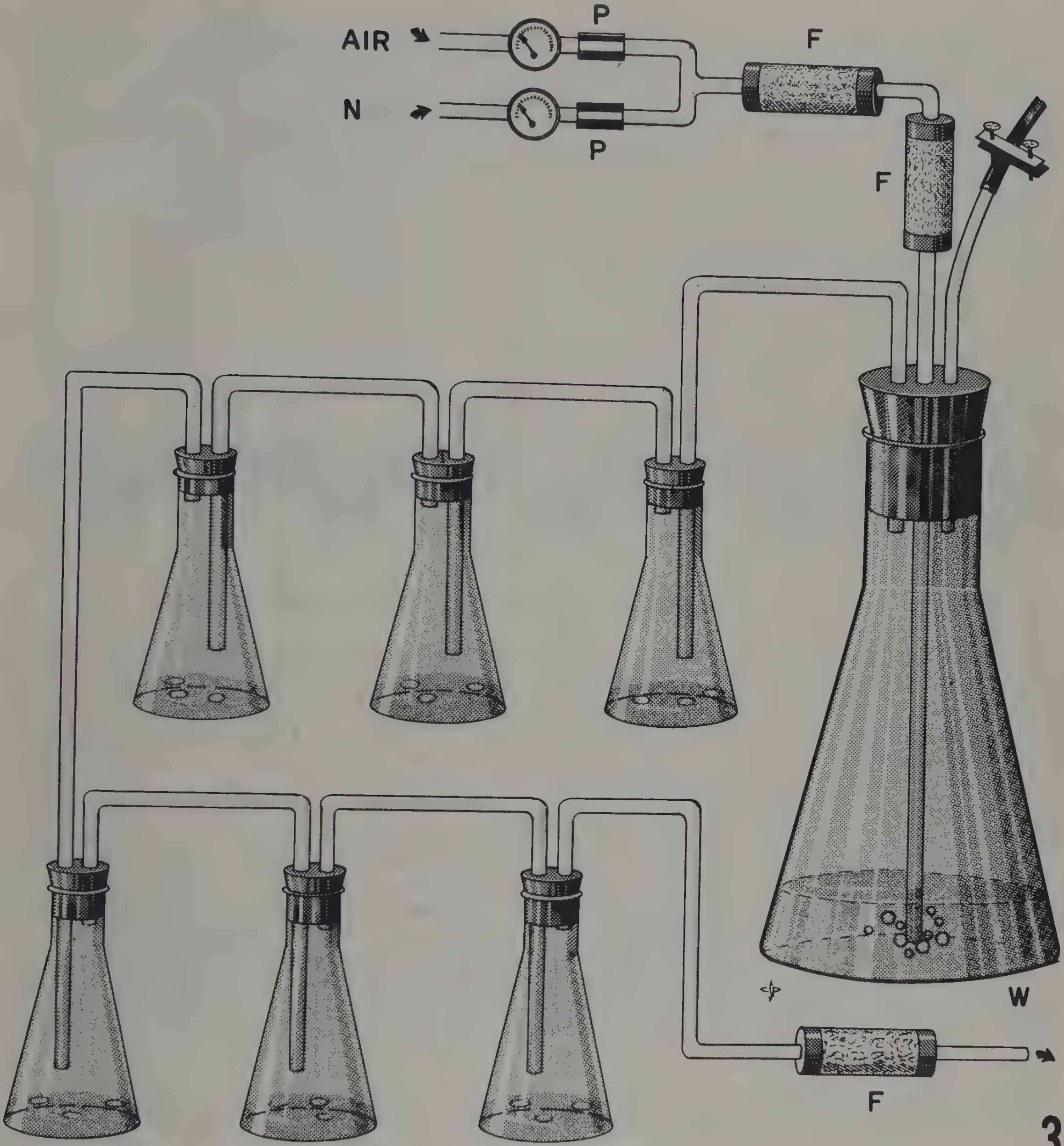

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FIGURE 1. A -- Aeration unit for aerating and shaking fungus cultures: F = cotton air filters; W = water in 500-ml Erlenmeyer flask for humidifying cultures; f = fulcrum on which unit rocks. B -- Aeration unit covered with wrapping paper prior to autoclaving.

FIGURE 2. Equipment for aerating and shaking fungus cultures: S = shaker which imparts rocking motion to units A, B, C, D, E, each with six cultures in Erlenmeyer flasks; f = fulcrum; N_2 = tank of compressed nitrogen; Air = tank of compressed air; M = oxygen meter; P = flow plugs; F = cotton air filters.

FIGURE 3. Detail of aeration unit: F = cotton air filters; W = water for humidifying cultures; P = flow plugs to regulate flow of gases.

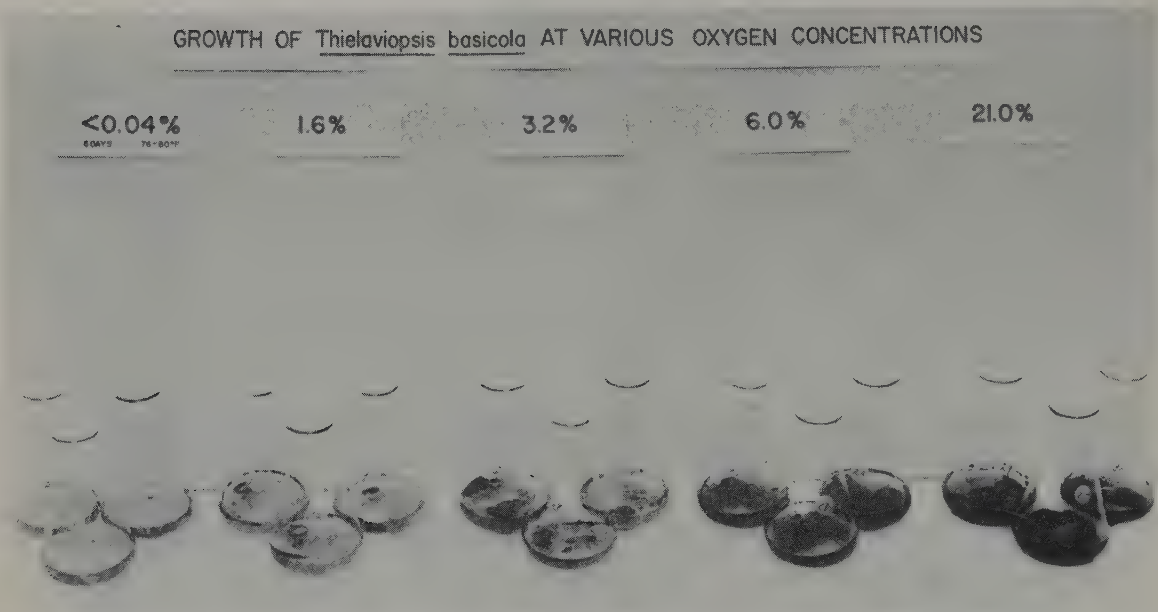
GROWTH OF Thielaviopsis basicola AT VARIOUS OXYGEN CONCENTRATIONS
<0.04%
6 DAYS
76-80°F
1.6%
3.2%
6.0%
21.0%

FIGURE 4. Effect of oxygen concentration on growth of Thielaviopsis basicola.

of the fungus on potato-dextrose agar. The tube delivering the gas mixture was raised to 4 cm above the surface to avoid foaming. Also, since it was found next to impossible to deliver the gas mixture at the same rate to all six replicates of cultures when arranged in parallel by means of a manifold, the replicates were connected in series so that the same volume of a given gas mixture passed over each culture. During the incubation period of 6 to 12 days the cultures were shaken with a rocking motion (Fig. 2).

Gas mixtures were obtained by metering compressed nitrogen and oxygen (or compressed air) through plaster of paris flow plugs into the same delivery tube leading to the six replicate culture unit. The percentage of oxygen in the mixtures was determined with a Beckman oxygen meter, concentrations in percentages used in the preliminary experiments being 0.0 to .04, 0.4, 1.6, 6.0, and 21.0. Dry weights of the mats of the three fungi studied, Phytophthora citrophthora, P. parasitica, and Thielaviopsis basicola (Fig. 4), were directly proportional to the concentration of oxygen in the gas mixtures supplied. Although the Phytophthora spp. appeared to stop growing soon after being placed in nitrogen (0 to 0.04% oxygen contaminant), they were viable when transferred to potato-dextrose agar after 8 days' exposure to this gas. Details of the results of the experiments will be published later along with data from relevant reports.

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SUMMARY OF REDUCTION IN YIELD CAUSED BY THE MAJOR COTTON DISEASES IN THE UNITED STATES FROM 1957 TO 1961

Compiled by the Cotton Disease Council, Committee on Disease Losses: Harlan E. Smith, Chairman; A. L. Smith, W. E. Cooper

Diseases	1957		1958		1959		1960		1961		Total loss	Average loss	
	Bales	%	Bales	%	Bales	%	Bales	%	Bales	%	bales	Bales	%
1. FUSARIUM WILT *Fusarium vasinfectum*	148,711	1.11	101,821	.74	158,583	.93	211,384	1.24	161,907	.94	782,406	156,481	.99
2. VERTICILLIUM WILT *Verticillium albo-atrum*	239,742	1.78	331,384	2.42	287,536	1.69	288,986	1.69	584,129	3.40	1,731,777	346,355	2.19
3. BACTERIAL BLIGHT *Xanthomonas malvacearum*	233,067	1.74	468,026	3.42	419,119	2.48	387,705	2.27	329,931	1.92	1,837,848	367,569	2.36
4. ROOT ROT *Phymatotrichum omnivorum*	140,845	1.05	198,718	1.45	272,681	1.61	312,703	1.83	364,617	2.12	1,289,564	257,912	1.61
5. ANTHRACNOSE BOLL ROT *Glomerella gossypii*	74,461	.55	24,502	.18	79,962	.47	73,345	.43	44,821	.26	297,091	59,418	.37
6. SEEDLING DISEASES *Rhizoctonia*, etc.	322,738	2.41	343,859	2.58	343,809	2.09	555,798	3.26	551,404	3.20	2,117,608	423,521	2.70
7. ASCOCHYTA BLIGHT *Ascochyta gossypii*	12,382	.09	10,281	.07	27,483	.16	20,784	.12	88,095	.51	159,025	31,805	.19
8. BOLL ROTS *Rhizopus*, etc.	275,961	2.06	322,240	2.35	380,109	2.24	372,906	2.19	295,311	1.71	1,646,527	329,305	2.11
9. ROOT KNOT *Meloidogyne* sp.	146,674	1.09	136,346	.91	193,050	1.13	308,979	1.81	288,374	1.67	1,073,423	214,684	1.32
10. OTHERS	16,432	.12	20,601	.15	107,773	.63	204,042	1.19	206,909	1.20	555,762	111,152	.65
TOTAL	1,611,013	12.00	1,957,778	14.27	2,270,110	13.43	2,736,632	16.03	2,915,498	16.93	11,491,031	2,298,202	14.49

COTTON DISEASE COUNCIL, COMMITTEE ON DISEASE LOSSES

PRELIMINARY STUDIES ON THE CONTROL OF NEMATODES IN OUTDOOR ROSE PLANTINGS

A. F. Schindler and T. J. Henneberry[1]

Abstract

Cynem and DBCP applied at two times the recommended rates failed to significantly reduce the plant-parasitic nematode population.

When the chemicals were applied at up to eight times the recommended rates there was a significant reduction in nematode populations.

The statistically significant reductions in nematode populations were not felt to be of practical significance.

It is suggested that soil type may influence the effectiveness and phytotoxicity of nematocides.

No plant injury was observed in any of the treatments.

INTRODUCTION

Control of insects and fungus diseases are important considerations in the culture of healthy, vigorous, high-yielding rose plants. Periodic applications of sprays or dusts are necessary to control spider mites, Japanese beetles, thrips, blackspot, and powdery mildew. These insects and fungi are readily seen on the aboveground portion of roses. Plant-parasitic nematodes, however, may remain unnoticed beneath the soil until plant damage occurs. Little information is available on effective materials and practical methods of application of use to the home rose gardener for the control of nematodes. Several genera of nematodes are commonly found in soil where greenhouse (8) or outdoor roses (2) are grown and three of these have been shown to cause extensive root injury. A species of dagger nematode, Xiphinema diversicaudatum, has also been shown to affect the growth and overall appearance of rose plants (9).

Chemical treatment for the control of nematodes by the home rose gardener is often desired in soil in which plants are growing. The number of nematocides that can be applied in the presence of some plants without phytotoxicity is limited. Two such materials are 1,2-dibromo-3-chloropropane (DBCP) and O,O-diethyl O-2-parazinyl phosphorothioate (Cynem).

DBCP has given excellent control of root-knot nematodes, Meloidogyne spp., on ligustrum, gardenia, and boxwood (3). Other investigators have reported control of root-knot nematode species with DBCP on strawberries (6).

Cynem has been reported as giving excellent control of root-knot nematodes on tobacco and may also have some value for the control of aphids and leafhoppers (4, 5). Sasser and Cooper (7) reported excellent control of the sting nematode, Belonolaimus longicaudatus Rau, on peanuts with Cynem. The nematode control was accompanied by increased yields. In the same report reduction of populations of thrips and leafhoppers were observed.

This paper presents the results of preliminary experiments conducted at Beltsville, Maryland in 1960 and 1961 to evaluate the nematocides DBCP and Cynem for control of nematodes in soil both before roses are planted and on established plantings.

MATERIALS AND METHODS

Preplanting soil treatments of each nematocide, in granular form, were applied in the first experiment on May 5, 1960, when the soil temperature was 65° F. The rates of application of DBCP were 8.8, 17.6, or 35.2 pounds of active ingredient per acre. Cynem was applied at the rates of 4, 8 or 16 pounds of active ingredient per acre. For both chemicals these rates were approximately one-half, one, and two times the recommended rates. The nematocides were distributed in holes drilled 6 inches deep and 12 inches apart over the 24-square-foot area of each plot; 11 days later plants were set in the field.

The soil was classified[2] as extremely variable, with Sunnyside sandy loam and Keyport

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[2] Soil test information was obtained from the Soil Testing Laboratory, University of Maryland, College Park, Maryland.

Table 1. Experiment No. 2, 1960-61. Average number of nematodes per sample from rose plots following treatment with granules or emulsion of DBCP or Cynem.

Chemical	Active ingredient/acre (pounds)	Granules	Emulsion
Untreated check		94 def*	113 def
DBCP	35.2	71 cde	36 ab
	70.4	44 bc	60 c
	140.8	21 a	38 b
Cynem	16	105 def	66 c
	32	70 c	64 c
	64	40 b	59 c

*Means not followed by the same letter are significantly different at the 5% level, according to Duncan's Multiple Range Test.

loam at various locations in the plots. Soil samples from the north and south ends of the plot were classified as fine sandy loam with pH of 4.2 and 4.8; very low and low MgO; and 2.9 and 2.4% organic matter, respectively. Both samples were rated medium for phosphate and potash. The experimental planting was arranged in a randomized block design of four replications. The data were analyzed and all differences are significant at the 5% level. Each plot consisted of six rose plants, three each of the varieties Peace and Helen Traubel. Plants were placed 2 1/2 feet apart in a plot with 5 feet between plots. The plants were mulched with 3 to 4 inches of sawdust, which was temporarily removed from the required areas whenever treatments were applied or soil samples taken. Applications of 5-10-5 fertilizer were made as needed.

Postplanting treatments were applied on June 29, approximately 7 weeks after planting, when the roses were established.

The failure of the treatments in the first experiment to reduce nematode populations effectively allowed the use of the same rose planting in a second experiment conducted in the fall of 1960 and spring of 1961.

In the second experiment soil treatments were applied on September 9, 1960, and arranged in an experimental design which made it possible to consider carryover effects of the previous treatments. DBCP and Cynem were applied as granules and emulsion concentrates. DBCP was applied at the rates of 35.2, 70.4, and 140.8 pounds of active ingredient per acre and Cynem at the rates of 16, 32, and 64 pounds of active ingredient per acre. The granules were applied as previously described. The emulsions were drilled into the soil to a depth of 6 inches with a hand-operated soil-fumigant applicator. Soil samples were taken and size of nematode populations determined on April 9, 1961.

For both experiments the effect of nematocide applications on nematode populations was assessed by taking four soil plugs, 3/4 inches diameter x 9 inches, from each plot. The soil plugs were thoroughly mixed in approximately 3 gallons of water and screened through 30- and 200-mesh screens. The nematodes were washed from the 200-mesh screen with water into a beaker, and treated as described by Schindler (10). The number of plant-parasitic nematodes in each sample was estimated by counting those in a quarter sector of a marked Syracuse dish.

RESULTS

In the first experiment preplanting or postplanting treatments of DBCP or Cynem did not result in a statistically significant reduction of the two plant-parasitic species found (*Hoplolaimus* n. sp. and *Xiphinema americanum* Cobb) and no plant injury due to chemical treatment was observed.

In the second experiment the nematode population was lower in plots treated with the high rate of granular DBCP (140.8 pounds active per acre) than in any of the other treated or check plots -- except those plots treated with the DBCP emulsion at 35.2 pounds of active ingredient per acre. All plots had lower nematode populations than the untreated checks (Table 1) -- except DBCP granules at the rate of 35.2 pounds of active ingredient per acre and Cynem granules at the rate of 16 pounds of active ingredient per acre.

Table 2. Experiment No. 2, 1960-61. Number of nematodes per sample from treated rose plots.

Chemical	Active ingredient/acre (pounds)	Nematodes per sample	Statistical significance*
Check		104	d
DBCP	35.2	53	a b c
	70.4	52	a b c
	140.8	29	a
Cynem	16	85	c d
	32	67	b c
	64	49	a b

*Means not followed by the same letter are significantly different at the 5% level, according to Duncan's Multiple Range Test.

When the total effect of a chemical treatment is considered (emulsions plus granules), plots treated with DBCP or Cynem at any of the rates tested had lower nematode populations than the untreated check with the exception of Cynem at the lowest rate of application (16 pounds per acre). However, except for the high versus the low rate of Cynem, no differences occurred between increased rates of application for either chemical (Table 2). No plant injury due to chemical treatment was observed.

DISCUSSION

Nematocides should reduce populations of plant-parasitic nematodes about 90% before they can be considered effective. Statistically significant reductions in nematode populations very often may not be of any practical significance because of the rapid buildup of the residual population.

The apparent practical ineffectiveness of the nematocides tested at even the high concentrations (eight times the recommended dosage) was unexpected. DBCP and Cynem have been shown by investigators to control nematodes to a practical level on other crop plants. However, Wilson, et al. (11), testing both chemicals, reported poor control of root knot, M. hapla Chitwood, on Caryopteris incana except where DBCP was used at the 300 pounds of 17.3% active granules per acre rate. These tests were conducted in light, sandy loam.

The soil type in the present experiments was classified as variable, containing both moderately well drained Sunnyside sandy loam and poorly drained Keyport loam. The reason for the poor nematode control in these experiments was not determined, but it has been demonstrated that soil type may have an effect on the efficiency of nematocides (1). Poor control may be due to chemical or physical soil factors or to a combination of the two factors. Undoubtedly the interactions of various soil types with various chemicals may be considerably different. In any tests involving soil fumigation, the type of soil should be determined and reported. A fumigant effective when used in one soil may prove ineffective in another type of soil. In addition, plant toxicity of a chemical may also vary considerably in different soils. In these experiments neither of the nematocides was toxic to the rose plants even at the maximum rates used.

At present, experiments are being conducted investigating the effects of different soil types on nematocide efficacy.

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EFFECT OF VOLUME AND VARIATION IN DROPLET SIZE OF SPRAY APPLICATIONS FROM AN AIR-CARRIER SPRAYER ON CONTROL OF THREE ALMOND DISEASES

Joseph M. Ogawa and Wesley E. Yates

Summary

Air-carrier spray applications were tested to determine the effect of operating techniques and application equipment. Applications of 0.85, 1.60 and 3.85 gallons per tree, and various degrees of atomization, did not alter the effectiveness of the sprays to control three almond diseases.

INTRODUCTION

The air-carrier type sprayer used in California orchards usually delivers high amounts of spray in large-size droplets. This type machine can be modified to deliver smaller amounts of spray in smaller droplet size. However, only a few growers have accepted the low volume (0.3 to 1.0 gallons per tree) concentrate-type applications, even though the principle was introduced in California some years ago. The present study is concerned with the effectiveness of equal quantities of ziram and captan, applied at various spray volumes and in various droplet sizes, in controlling three almond diseases.

MATERIAL AND METHODS

The plot consisted of 130 almond trees (*Prunus amygdalus*) cultivar 'Ne Plus Ultra,' with two buffer rows of the cultivar 'Nonpareil' between each test row. A Latin square design was used for the spray treatments and five single trees were distributed throughout the plot to serve as control. The trees were sprayed with 67% ziram at the rate of 0.08 pounds per tree at both pink-bud and petal-fall stages of bloom. Five weeks after petal fall they were resprayed with 0.08 pounds of 50% captan per tree. The sprays were tested against Coryneum blight (*Coryneum beijerinckii* Oud.), scab (*Cladosporium* sp.) (1), and leaf blight (*Hendersonia rubi* Sacc.) (2).

Applications were made with an air-carrier sprayer constructed by the Bear River Welding and Supply Company, Wheatland, California (Fig. 1). The machine had 37,000 cfm air capacity, a 400 gallon tank, a 70 gpm centrifugal pump, and a 40 gpm positive displacement pump. The droplets of large, medium, or small size were attained by core size of the whirl plate, number of nozzles, and by spray pressure. The volume at these droplet sizes was regulated by the number of nozzles, giving 0.85, 1.60, or 3.85 gallons of spray for each tree. All applications were made at a speed of 2.0 miles per hour. The spray distribution pattern around the air discharge section was adjusted to give thorough coverage of the tree,- and was identical for all of the above tests. Table 1 shows the manner in which the above conditions were developed.

RESULTS

Statistical comparisons, using Duncan's multiple range test, revealed no significant differences among the five treatments in the control of Coryneum blight, scab, or leaf blight (Table 1). However, significant differences in disease incidence were shown between each treatment and the control, except for treatment C (4 gallons per tree of medium spray droplet) on scab control.

A sample of sprayed leaves from the center of a tree in each treatment is shown in Figure 2. The only noticeable effect was the discrete deposits formed by 0.85 (A) to 1.60 (B) gallons of spray applications per tree.

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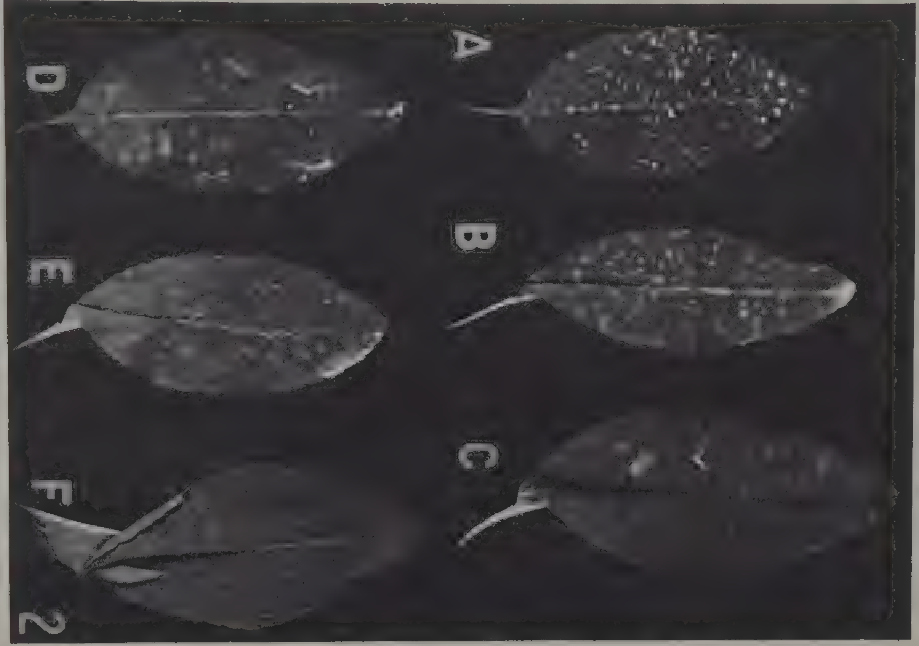
Table 1. Effect of spray volume and droplet size on disease incidence of Coryneum blight, scab, and leaf blight diseases of almond.

Treatment	Gallons per tree	Droplet size	Psi	No. and pattern of nozzles at each outlet		Core size	No. of whirl plates used: Orifice sizes D4	No. of whirl plates used: Orifice sizes D5	% diseased nuts: Coryneum blight	% diseased nuts: scab	Av. no. leaf blight per 20-inch shoot
A	0.8[a]	medium	50	12	•	45	4	8	14.0 a*	46.2 a	1.07 a
B	1.6	medium	50	24	••	45	8	16	16.0 a	43.8 a	1.15 a
C	4.0	medium	50	60	⁙	45	20	40	15.5 a	55.8 ab	0.91 a
D	4.0	large	65	36	⋱	56	12	24	21.8 a	43.8 a	1.06 a
E	4.0	small	530	12	•	56	4	8	13.3 a	33.6 a	1.07 a
F (control)									56.6 b	72.0 b	3.66 b

[a] Deviations from the calculated dosages were A, +0.05; B, 0.00; C, -0.15; D, -0.15; and E, +0.05 gallons per tree.

*Duncan's multiple range test at 5% level. Treatments having the same letter are not significantly different.



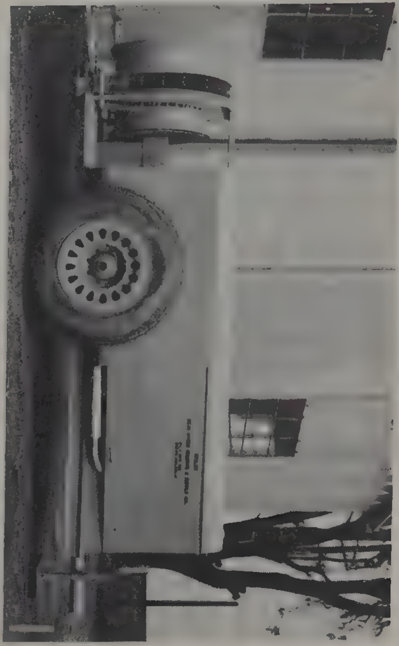


FIGURE 1. Side view of air-carrier sprayer developed by the Bear River Welding and Supply Co., Wheatland, California. Tank -- 400 gallon capacity, pumps a (70 gpm at 86 psi b) 40 gpm Meyers at 600 psi positive pressure, blower -- two 28 inch Robinson E-5 axial flow fans with designed capacity of 37,500 cfm at 25,000 rpm and engine -- Ford heavy duty V-type 8 cylinder industrial.

FIGURE 2. Pattern of spray deposit on leaves. Gallons of medium sized droplet spray per tree: A, 0.8; B, 1.6; and C, 4.0. Four gallons per tree of spray droplet sizes of: small E, medium C, and large D. Control is designated as F.

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USE OF NYLON MESH IN STUDIES OF SOIL FUNGI

K. M. Old[1] and T. H. Nicolson[2]

The fungistatic effect of soil has been extensively studied using fungal spores (1, 2) but few investigations have been made using established mycelium. Lockwood (3, 4) observed lytic effects of soil on plate cultures of mycelium.

Nylon mesh was used by Waid and Woodman (5) to observe hyphal growth and activity in field soils. During our studies on antagonism exhibited by a Streptomyces sp. to Rhizoctonia solani Kuehn, we found that this material offered a convenient way of introducing the fungal mycelium into soil, and allowing subsequent recovery. Various patterns of mesh were tried, the most suitable being monofilament with approximately 400 squares per square centimeter. When the fungus was grown on this material its hyphae interwove through the mesh, and were retained even after removal from the soil, when adhering soil particles were washed off. Nylon has the advantage of being virtually biologically inert (5) and can be autoclaved without damage. The following techniques were developed using the mesh.

The intent was to observe lytic effects of the Streptomyces sp. on R. solani. Discs of potato-dextrose agar 2 mm in diameter bearing R. solani were cut from the advancing edge of a 4-day-old colony. These were placed onto the center of sterilized nylon mesh 1.5 cm square, applied to a potato-dextrose agar (PDA) surface, and the fungus allowed to grow across the mesh for 3 days. The squares were then stripped off the agar and placed about 1 inch below the surface of soil previously inoculated with the Streptomyces sp. The soil was contained in wide-mouthed quart jars and incubated at 25° C. After a certain period the meshes were recovered, the adhering soil particles washed off, and after staining with cotton blue, the meshes were mounted in lactophenol on a glass slide. The squares of the mesh were used as standard fields for scoring lysis under the microscope (Fig. 1).

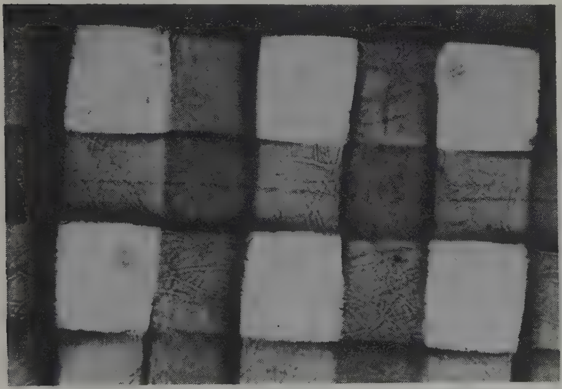

FIGURE 1. Photomicrograph of mesh showing mycelium of R. solani, after recovery from soil. Approx. X75.

In a later study, the effect of Streptomyces sp. upon rate of extension of R. solani across the mesh was studied. Strips of mesh, 6 x 1.5 cm, were prepared and autoclaved. One strip was placed into each soil jar and a disc of PDA, diameter 1.25 cm, bearing R. solani placed on the center of the strips. The strips were then covered with soil to a depth of 1 inch and the jars incubated at 25° C. After recovery from the soil, the agar disc was carefully removed, the strip washed, stained, and mounted in lactophenol. The distance that the mycelium had traveled either side of the mesh was measured, and any effect of the Streptomyces sp. noted.

The possibilities of nylon mesh in investigations of soil fungi are considerable, and many valuable techniques could be devised employing this material in several fields of study.

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A REPORT OF THE OCCURRENCE OF CAMELLIA FLOWER BLIGHT IN MISSISSIPPI

J. H. Cochran[1]

In the latter part of May our inspector, Mr. Thad. I. Pigott of McComb, mailed me some specimens of old flowers from camellia plants that he suspected of having camellia flower blight. Upon checking these specimens, I felt sure the dark objects at the base of the petals were sclerotia of camellia flower blight. Since these sclerotia had never been directly observed, the specimens were sent to Dr. A. G. Plakidas, Professor Emeritus of Louisiana State University, who has had more experience with the disease. He confirmed the disease to be camellia flower blight.

The locality where this disease was found has since been visited and casually surveyed and the disease found to be present over the entire city of McComb, a city of approximately 12,000 people. It is also known to be present in Magnolia, the county seat of Pike County, located 6 miles south of McComb.

The State Plant Board will initiate the control measures and quarantines necessary to restrict the spread of the disease.

MISSISSIPPI STATE PLANT BOARD, STATE COLLEGE, MISSISSIPPI

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THE SPREAD OF LIMA BEAN DOWNY MILDEW (PHYTOPHTHORA PHASEOLI) STRAIN "B" IN NEW JERSEY

R. E. Wester and Tejpal S. Gill[1]

A new race of downy mildew of lima bean caused by *Phytophthora phaseoli* Thaxt. known as strain "B" was first reported by Wester and Jorgensen[2] in 1958 on the Newkirk Farm but was not active in 1959. From this field in 1960 it spread slightly north of Pittsgrove and to six other locations south to the Research Center of Seabrook Farms Company. Strain "B" was not found south or west of Seabrook, New Jersey, or other areas of New Jersey. In 1961 this strain was not reported in any of the above or new locations.

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THE PLANT DISEASE REPORTER

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Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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CONTENTS

1. A new disease of American spearmint caused by tobacco ringspot virus
W. J. STONE, et al. 623

2. A lily ringspot virus from Georgia
PHILIP BRIERLEY 625

3. Easter lilies freed from cucumber mosaic virus by scale propagation
PHILIP BRIERLEY 627

4. Preservation of activity of some stone fruit viruses
H. C. KIRKPATRICK 628

5. Yields and flavor of potatoes and carrots on plots receiving annual soil treatments
BERT LEAR, et al. 631

6. Treatments for eradication of Ditylenchus dipsaci in cloves of garlic
BERT LEAR and D. E. JOHNSON 635

7. Cynem -- An effective control for sting nematodes on peanuts
W. E. COOPER and J. N. SASSER 640

8. Pathogenicity of Phytophthora palmivora in the Orchidaceae
RICHARD B. HINE 643

9. Control of Aphanomyces root rot of peas in the greenhouse with methionine and norleucine
G. C. PAPAVIZAS and C. B. DAVEY 646

10. Chemical control of Rhizoctonia solani and Sclerotium rolfsii of snap beans in Boca Raton, Florida, 1962
HUEY I. BORDERS 651

11. Chemical control of bacterial and Cercospora leaf spots and Phytophthora blight of pepper on south Florida sandy soils
HUEY I. BORDERS 652

12. Ramularia schulzeri on birdsfoot trefoil in New York
RICHARD E. FORD 655

13. Yield reductions and stalk rot severity in corn hybrids under severe Helminthosporium leaf blight conditions in Pennsylvania
C. C. WERNHAM, et al. 657

14. Rhizoctonia blight of tall fescue grass
E. S. LUTTRELL 661

15. Verticillium wilt of safflower in the United States -- A potential problem
D. E. ZIMMER 665

16. Distribution, prevalence and importance of some cantaloup virus diseases in southwestern Arizona
MERRITT R. NELSON, et al. 667

17. Control of Alternaria blight of rape and mustard by griseofulvin
AKHTAR HUSAIN and RAM NATH THAKUR 672

18. A comparison of isolation methods for Fomes annosus
FLOYD F. HENDRIX, Jr. and E. GEORGE KUHLMAN 674

19. Oak anthracnose in Mississippi
G. K. PARRIS and JOHNNIE BYRD 677

20. Unusual severity of leaf-blister on certain species of oak in Mississippi
G. K. PARRIS 682

21. New hosts and nonhosts of reniform nematode
WRAY BIRCHFIELD and L. R. BRISTER 683

22. Intracellular inclusions observed in epidermal strips from foliage of cadang-cadang infected coconut trees in the Philippines
D. B. PROTACIO 686

23. Efficacy of fall applications of copper and organic fungicides for the control of Coryneum blight of peach in California
HARLEY ENGLISH and J. R. DAVIS 688

24. Mosaic, a new virus disease of Kalanchöe flammea
HARRY C. BURNETT and RAY A. LONG 692

25. Additional hosts of the fig mosaic virus
HARRY C. BURNETT 693

A NEW DISEASE OF AMERICAN SPEARMINT CAUSED BY TOBACCO RINGSPOT VIRUS[1]

W. J. Stone, G. I. Mink, and G. B. Bergeson[2]

Numerous severely stunted and rosetted American spearmint (Mentha spicata) plants were observed in a northern Indiana muck field during the summer of 1961. Diseased plants were conspicuous because of their reduced size, proliferated growth habit, and deformed leaves (Fig. 1). When healthy and diseased spearmint plants were transplanted into 5-inch pots and held in the greenhouse, the healthy plants grew normally throughout the summer, whereas diseased plants produced little or no new growth and eventually died.

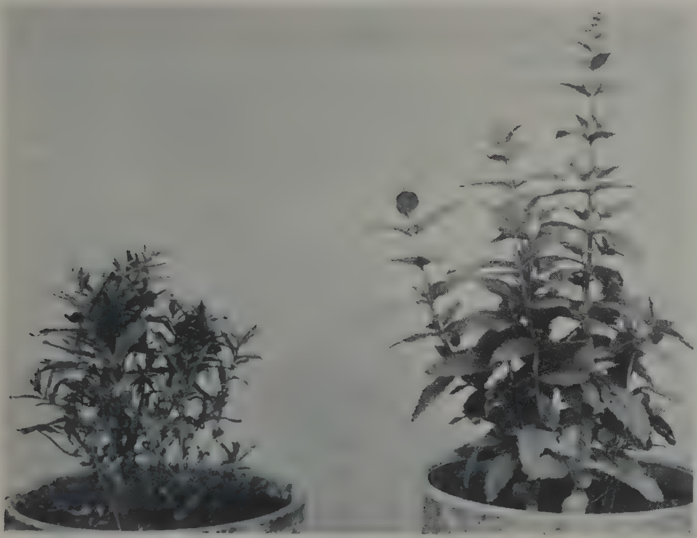

FIGURE 1. Left -- American spearmint plant infected with tobacco ringspot virus showing rosetted growth and deformed leaves. Right -- Healthy plant.

Tip leaves from diseased spearmint were macerated in 0.01 M potassium phosphate buffer at pH 7.0 and used to inoculate primary leaves of bean (Phaseolus vulgaris var. Bountiful). Within 7 to 10 days a severe necrotic shock reaction similar to that caused by tobacco ringspot virus (TRSV) developed on young trifoliate leaves. Subsequent inoculations induced symptoms diagnostic of TRSV on the following hosts: cowpea (Vigna sinensis var. Ramshorn), tobacco (Nicotiana tabacum var. Connecticut Havana 423), cucumber (Cucumus sativus var. National Pickling), jimson weed (Datura stramonium), Chenopodium amaranticolor, and soybean (Glycine max var. Gibson). Leaves from healthy spearmint plants tested in the same way induced no symptoms on the herbaceous indicators. The virus obtained from mint and cultured in cowpea reacted with tobacco ringspot antiserum purchased from Microbiological Associates, thus confirming the diagnosis made on the basis of host range.

TRSV could be obtained from all spearmint plants visibly diseased, but attempts to introduce TRSV into healthy mint plants or cuttings by mechanical means were unsuccessful. Macerated tissue from TRSV-infected mint, bean, cowpea, and tobacco plants was applied by rubbing to carborundum-dusted spearmint leaves, stems and roots, by dipping roots and cut stems into the inoculum, and by grafts. Inoculated plants were grown in the greenhouse, and subsequent indexing of these plants on bean indicated that systemic infection did not occur.

In the field, TRSV-infected plants were localized in two relatively small areas. In each case, soil samples indicated high populations of Xiphinema americanum Cobb, a known vector[3] of TRSV. Adjacent areas of only healthy mint plants contained few if any X. americanum. Bean and cucumber seedlings grown in muck soil collected from the root zone of infected mint plants developed typical TRSV symptoms within 1 to 3 weeks in the greenhouse when grown in insect-proof cages.

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[1] Journal paper number 1952 of the Purdue University Agricultural Experiment Station.

[2] Graduate student and Assistant Professors, respectively, Department of Plant Pathology, Purdue University, Lafayette, Indiana.

[3] Fulton, Joseph P. 1962. Transmission of tobacco ringspot virus by Xiphinema americanum. Phytopathology 52: 375.

Handpicked lots of 600 and 300 X. americanum taken from the root zone of infected plants transmitted TRSV to cucumber. This is in agreement with the results obtained by Fulton[3]. A more detailed report on the nematode-virus relationship will be reported elsewhere.

Fourteen healthy American spearmint cuttings were rooted and grown for several months in the original field infested muck soil. Although none of these plants developed obvious symptoms, one of the 14 was found to contain TRSV in the tip leaves after 2 months. The aboveground portions of all 14 plants were removed after 5 months and growth from rhizomes observed. Two rhizomes produced plants typical of those in Figure 1 and contained TRSV. In a similar experiment, rhizomes from one of five cuttings rooted in infested soil for 6 weeks developed typical symptoms. The data suggest that following initial infection by TRSV through the roots, American spearmint plants remain symptomless even though the virus may become systemic. Symptoms are produced only on plants that develop from infected rhizomes.

Rhizomes collected from TRSV-infected plants in the field during 1961 and 1962 indicated that the virus successfully overwintered in the rhizomes.

No typical symptoms have been observed in the field or greenhouse on scotch spearmint (Mentha cardiaca). However, when a limited number of clones of unknown origin from the greenhouse were indexed, they were found to contain TRSV. Rhizome growth from the infected plants was normal even though TRSV could be isolated. The indications are that TRSV is probably latent in scotch spearmint.

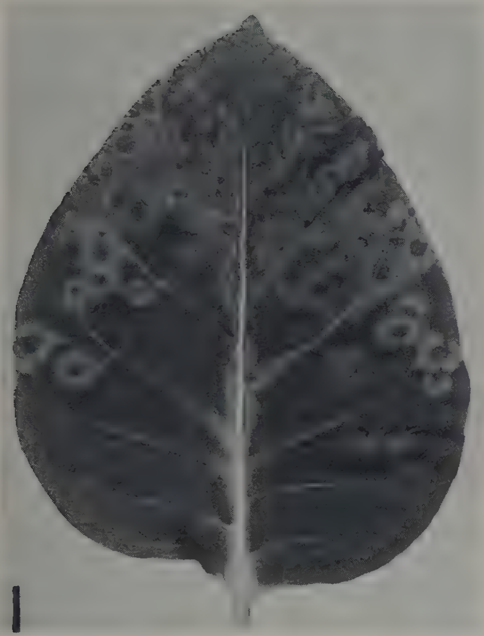
PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION, LAFAYETTE, INDIANA

A LILY RINGSPOT VIRUS FROM GEORGIA

Philip Brierley[1]

In 1960 I received from Tifton, Georgia Easter lilies (Lilium longiflorum) of the 'Georgia' cultivar showing conspicuous yellow mosaic patterns. Sap transfer from these to plants of the seed-propagated cultivar 'White Queen' developed, 11 days later, similar yellow mosaic symptoms which failed to mask at flowering time as the lily mottle virus generally does.

In 1961 sap transfers from the 'Georgia' cultivar to 'Samsun' (Fig. 1) and 'Kentucky 35' (Fig. 2) tobaccos (Nicotiana tabacum) yielded a yellow ringspot virus with local to partly systemic symptoms, sometimes resembling a yellow cucumber mosaic virus (CMV) such as CMV 6.



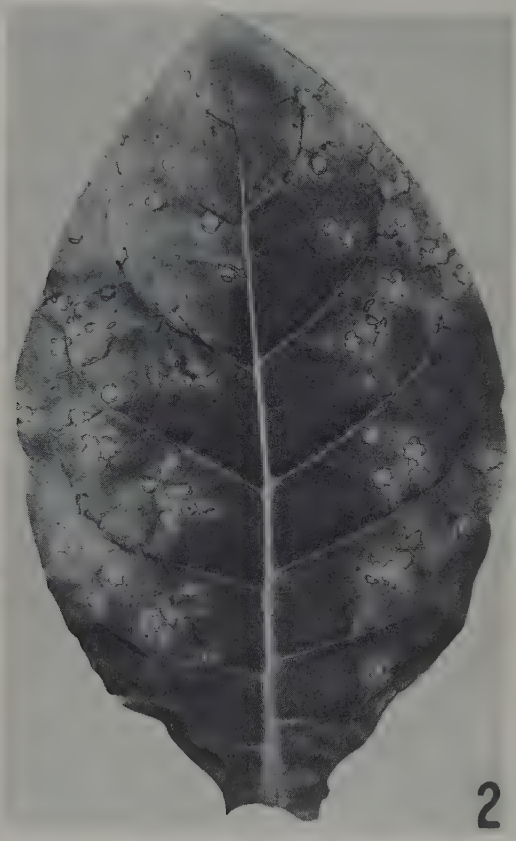


FIGURE 1. Lily ringspot symptoms in 'Samsun' tobacco.
FIGURE 2. Lily ringspot symptoms in 'Kentucky 35' tobacco.

No infection occurred in Datura stramonium, Dianthus barbatus, Capsicum annuum, Callistephus chinensis, Rudbeckia hirta, or four varieties of beans (Phaseolus vulgaris). 'Bonny Best' tomato (Lycopersicum esculentum) developed no symptoms but the virus was recovered from this. Chenopodium amaranticolor and 'Blackeye' cowpea (Vigna sinensis) expressed local lesions similar to those produced by CMV. Nicotiana clevelandii showed white etch symptoms, and N. glutinosa yellow spots that became systemic and partly confluent. 'Improved Long Green' and 'Marketer' cucumbers (Cucumis sativus) developed white dot local lesions, followed by systemic chlorotic spots. Gomphrena globosa expressed red ring local lesions with gray centers, and later systemic yellow blotches.

After repeated transfers to 'Samsun' tobacco, physical properties were determined. The virus source was 'Samsun' tobacco and indicator plants 'Samsun' and Chenopodium amaranticolor. Heat inactivation was 60° to 65° C, dilution end point 10^{-3} to 10^{-4}, and aging 1 to 2 days at about 25°.

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Like CMV (1) the lily ringspot virus can be eliminated from 'White Queen' lilies by propagation from outer scales. Fifteen of 16 inner and all four outer scales were free from ringspot virus but none was free from the lily mottle virus introduced into the 'White Queen' cultivar at the same time. The plants were inoculated in November 1960 and scaled in August 1961. Thus far, only CMV and lily ringspot viruses have been eliminated by scaling lilies. After elimination of lily ringspot virus, the 'White Queen' propagules were also free from the yellow mosaic pattern.

The lily ringspot virus is similar, and perhaps identical, to that described in 1950 by K. M. Smith (2). Smith described his virus as common in hybrid lilies from the United States but did not mention _L. longiflorum_, the only species of _Lilium_ in which I have seen the present virus. He succeeded in transmitting his virus with _Myzus persicae_ (Sulz.), but this aphid failed to move the present virus from tobacco to tobacco in a test by Floyd F. Smith. Both K. M. Smith and I failed to get satisfactory cross protection tests with the ringspot virus versus a tester strain of CMV. Inasmuch as lily ringspot virus is not fully systemic, it is poorly adapted to such tests.

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EASTER LILIES FREED FROM CUCUMBER MOSAIC VIRUS BY SCALE PROPAGATION

Philip Brierley

Cucumber mosaic virus (CMV) is an essential component of the fleck complex of Easter lily, *Lilium longiflorum*. Hence elimination of CMV eliminates the fleck symptoms. Scale propagation will eliminate this virus from a proportion of the scales of recently infected stocks, although it has no effect upon the lily mottle virus introduced into the source lily at the same time. This is demonstrated by indexing the fleck-free scales on *Lilium formosanum*, a sensitive indicator for the lily mottle virus.

Table 1 shows the proportion of CMV-free scales to vary widely with different stocks of Easter lily. Low yields of CMV-free scales, as in Breuille Creole may indicate infection of longer duration. High yields, as in White Queen, involve an unusually virulent strain of CMV, and suggest that more virulent strains are less invasive. This virulent CMV actually invaded only four of nine stem bulblets when these were separated and potted separately some 18 months after infection.

Table 1. Easter lilies scaled to eliminate CMV.

Cultivar	Disease	Date infected	Date scaled	Number CMV-free over total scales: outermost	next inner	total	CMV-free (%)
Beltsville grown sdlgs	CMV	?	10/17/59	30/32	----	30/32	94
Creole 2658	Fleck	1/19/60	9/19/60	1/1	1/3	2/4	50
Creole 2658	Fleck	1/19/60	8/10/61	3/3	----	3/3	100
Ace	Fleck	?	9/19/60	7/7	3/6	10/13	77
Breuille Creole	Fleck	?	9/19/60	14/32	21/42	35/74	47
Georgia	CMV	?	9/19/60	9/11	20/22	29/33	88
White Queen	CMV	2/27/61	8/10/61	7/8	13/13	20/21	95
White Queen	Fleck	1/12/60	6/14/61	15/15	----	15/15	100
Sum				86/109	58/86	144/195	---
Percent				79	67	74	

Because scaling yields a varying number of CMV-free propagules, the method is adapted to salvaging valued cultivars that have become contaminated rather than to commercial practice. Thus far this technique has been applied to Easter lilies only, but garden lilies would probably respond also. Because garden lilies do not develop fleck, they would need to be indexed in advance to determine the presence of CMV and again after scaling to determine its absence. When flecked Easter lilies are scaled, the elimination of fleck symptoms gives assurance that CMV is absent.

The initial suggestion for the scaling technique came from heaps of current-season-fleck Easter lilies discarded from southern Louisiana fields. In the following year these heaps of discards were covered with green scale propagules, all free from fleck. Apparently, the immature stems and inner scales withered away, but the mature outer scales that propagated freely had never been reached by current-season CMV infection.

Evidently CMV moves slowly through the basal plate of Easter lilies, whereas the lily mottle virus travels faster over the same path, although the two viruses move passively. Such differences in rate of movement may arise from the fact that CMV has necrotic tendencies as shown by fleck production, whereas the mottle virus seems to have none. Even slight necrosis in the basal plate might account for the observed slower rate of progress.

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PRESERVATION OF ACTIVITY OF SOME STONE FRUIT VIRUSES

H. C. Kirkpatrick

Summary

Stone fruit ringspot virus activity has been preserved for 5 years in cucumber tissue vacuum-dried over silica gel before freezing. An alternative method of drying cucumber tissue by direct contact with silica gel before freezing has no apparent detrimental effect upon infectivity of ringspot virus. Ten other Rosaceous viruses have been successfully preserved by vacuum drying.

INTRODUCTION

An isolate of stone fruit ringspot virus (RSV) has been used for more than 10 years in chemotherapy, purification, and host-range studies. This work has required a stock culture of this virus to be inoculated at 1- to 2-week intervals. Isolates of related and non-related stone fruit viruses accumulated make maintenance of such stock cultures burdensome.

RSV infectivity is lost when leaves are frozen (5), in expressed sap at low temperatures (1, 5, 10), or in purified preparations after intervals of generally less than 60 days (2, 5). McKinney (6) reported that the activity of cucumber mosaic virus was maintained more effectively by drying diseased tissue over $CaCl_2$ at 1° to 2° C than by drying diseased tissue at room atmosphere or over $CaCl_2$ at room temperature. Later reports (7, 8, 9) substantiated that virus survival was effective when plant tissue was desiccated at temperatures just above freezing. The purpose of this report is to present preservation methods that have been effective primarily with stone fruit ringspot virus.

METHODS

Infected tissue for all preservation tests was harvested 7 to 14 days after inoculation, and only good symptom-expressing tissue was used. Quantitative assay of the virus was based on a standardized gauze pad inoculation procedure (3) or on an airbrush inoculation procedure (4). All dilutions were on a fresh-weight basis.

RESULTS

Infected cucumber foliage kept over silica gel at 4° C under vacuum in a desiccator loses up to 89.4% of the fresh weight as moisture within 7 to 14 days. Tissue is placed in a small water- and air-tight container after drying and frozen at -22°. RSV infectivity has been preserved in this manner for nearly 6 years. Table 1 is a record of the retention of infectivity showing a gradual infectivity loss throughout the storage period of a ringspot isolate harvested February 15 and frozen on February 28, 1956.

Other virus isolates preserved in this manner for various periods and currently infective and the host tissue in which they are preserved are as follows:

4 years	2 RSV isolates in cucumber (Chicago Pickling)
2 years	2 RSV isolates in cucumber (Chicago Pickling)
1 year	1 unidentified raspberry virus isolate in *Gomphrena globosa*
1 year	1 unidentified raspberry virus isolate in *Zinnia elegans*
1/2 year	1 unidentified raspberry virus isolate in bean (California small white)
1/2 year	1 unidentified raspberry virus isolate in *Solanum miniatum*
1 year	4 prune dwarf virus isolates in squash (Buttercup)
1/2 year	2 unknown apple virus isolates in bean (California small white)

A more simple procedure, the direct-contact method, for preserving virus infectivity in tissue has been to layer cucumber cotyledons in dry silica gel. This procedure was tested only recently to determine the effect of silica gel contact on the virus activity of the tissue. Table 2 is a record of the effectiveness of this drying method.

DISCUSSION

Some stone fruit viruses are so unstable that they are immediately inactivated unless the tissue is dried or partially dried before freezing occurs. This is borne out by the results at

Table 1. Infectivity retention of one sample of dry-frozen RSV at three dilution levels as shown by mean number of lesions per cucumber cotyledon from two inoculation methods for the period February 28, 1956 to September 21, 1961.

Date tested	Gauze pad inoculation			Airbrush inoculation		
	1:20[a]	1:30	1:60	1:20	1:30	1:60
2/15/56 (Before drying)	---	9.0	---			
2/21/56 (Before freezing)	---	7.9	1.0			
2/28/60	7.7	4.9	0.3			
11/28/60	5.3	2.3	1.5	---	8.8	---
9/8/61	0.7	1.1	0.9	2.2	1.6	0.8
9/21/61	1.7	0.4	0	5.8	1.5	0.3

[a] Dilution of the preparation is on a fresh-weight basis. Buffer equal to original moisture added before the dilution.

Table 2. Infectivity retention of one sample of RSV infected tissue before drying and dried at three storage temperature levels as measured by mean number of lesions per cotyledon on cucumber test plants.

Treatment (° C)	Time after harvest (days)	Dilution of inoculum[a]		
		1:30	1:100	1:300
Check, not dried	0	4.6	0.4	0.1
20° - 25°	30	4.2	0.5	0.03
4° - 5°	36	0[b]	0	0
-22°	46	1.9	0.2	0.03

[a] Dilution of the preparation is on a fresh-weight basis. Buffer equal to original moisture is restored before the dilution is made.

[b] Moisture from atmosphere in cold room depleted silica gel drying capacity such that cotyledons were not completely dried when the infectivity was tested.

4° - 5° C (Table 2). While direct comparison of infectivity of tissue vacuum-dried over silica gel with the quick-dry method of layering in silica gel was not made, the quick-dry procedure removed tissue moisture three to four times as quickly. No apparent detrimental effect upon infectivity occurred from the silica gel.

The assurance that dried and frozen stone fruit virus material is infective for long periods should facilitate the exchange or the storage of these virus cultures.

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YIELDS AND FLAVOR OF POTATOES AND CARROTS ON PLOTS RECEIVING ANNUAL SOIL TREATMENTS

Bert Lear[1], W. F. Mai[2], M. B. Harrison[2], and H. S. Cunningham[2]

Summary

Nine chemicals were applied to replicated plots to determine their influence on yields and flavor of potatoes and carrots. A single dosage equivalent to that applied in 10 years was made for each material at the start of the experiment. Annual rates were applied to other plots at yearly intervals for 3 years. No annual dosage of any chemical resulted in a significant reduction in yield. Both crops were significantly reduced on plots receiving one application of 5000 pounds of D-D, Dowfume N, and dichlorobutenes. This reduction persisted for 1 year. Off-flavor was detected only in the 5000-pound rate for D-D and Dowfume N after 1 year, but not after 2 years.

With the amendment in 1959 to include nematocides under the Federal Insecticide, Fungicide, and Rodenticide Act, the need for data on residues, including any causing changes in flavor, becomes urgent. It seems desirable, therefore, to report data obtained from experiments conducted beginning in 1949. The purpose of these tests was to study the relation between the persistence in soil of nematocides and the yields and edible qualities of potatoes and carrots grown in soil previously treated with these chemicals.

The experiment was located at the Long Island Vegetable Research Farm, Riverhead. The taste tests were run at Ithaca with the cooperation of Dr. Alice M. Briant of the Department of Food and Nutrition, College of Home Economics.

In April 1949, an area classified as Sassafras silt loam 200 by 204 feet was divided into strips 20 feet wide. The proposed test crops were planted in each strip to index the experimental area as to productivity and any fungus or nematode infestations present. Two rows of potatoes (Katahdin) were planted in April. In July, three rows of carrots (Nautis) were sowed and three rows of cauliflower (Efurt) were set in each plot. Records were made of all fertilizer and spray applications to the plots. In September each strip was divided into plots 17 feet long. The potatoes were dug and yields per plot determined. Note also was made of amounts of scab, Rhizoctonia and wire worm injury. The cauliflower plants were dug and the roots scored for club-root infection. The carrots were dug and roots scored for degree of root-knot nematode damage and total yields per plot determined.

After indexing, the entire area was prepared for treatment by discing in two directions. Three replicates of treatments involving nine chemicals (Table 1) were applied to plots 17 x 20 feet. All liquids were injected 6 inches deep on 10-inch staggered centers by means of handguns. The dry materials were spread on the surface and raked into the soil. An attempt was made to include all chemicals recommended for application to the soil at the time the experiment was begun in addition to some of the newer materials which might be adopted later. Where a fumigant[3] contained more than one chemical, an attempt was made to include individual components as separate treatments. The dosages employed included at least one yearly recommended dosage and an amount normally applied in 10 years which was to be applied only once, in the fall of 1949. Three weeks after treatment, a cover crop of Ambruzzi rye was sowed in all plots. All annual applications were repeated in the fall of 1950 but only eight were selected, on the basis of the 2 previous years' experience, for application in 1951.

The same crops were planted each spring on the approximate dates utilized to index the experimental area. In the fall of each year, usually September, the carrots and potatoes were dug and yields recorded. Prior to taking yields, plants in 1 foot at each end of each plot were removed leaving plots 15 feet long for which yields were determined. Thus, a 2-foot buffer strip between all plots was provided. The potatoes were graded over a hand grader and yields given are in bushels of U. S. No. 1 tubers. In 1950 carrots were topped prior to weighing but not in 1951 and 1952. Records of the amounts of root-knot damage and diseases present were

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[2] Professor, Associate Professor, and Emeritus Professor, Department of Plant Pathology, Cornell University, Ithaca, New York.

[3] See Table 6.

Table 1. Summary of taste tests on potatoes grown in 1950 in plots treated in 1949 with various soil fumigants and other chemicals, each at dosages recommended for annual applications and at single dosages based on amounts normally applied in 10 years.

Material	Dosage (lb/acre)	No. of opinions[a]	% of opinions[b] rating samples as indicated off-flavor: None	Weak	Moderate	Strong	Objectionable
None (unknown check)	---	254	87	8	2	2	1
D-D mixture	250	34	88	9	0	3	0
D-D mixture	500	33	73	12	12	3	0
D-D mixture	5000	37	54	11	8	19	8
Dowfume N	250	37	55	32	8	5	0
Dowfume N	500	35	77	14	6	0	3
Dowfume N	5000	38	15	24	24	21	16
Ethylene dibromide	25	34	70	24	3	3	0
Ethylene dibromide	50	33	76	15	6	0	3
Ethylene dibromide	500	36	63	17	14	0	6
Dichlorobutenes	250	32	66	16	9	6	3
Dichlorobutenes	500	33	43	27	12	9	9
Dichlorobutenes	5000	-	-	-	-	-	-
Propylene dichloride	250	23	77	18	5	0	0
Propylene dichloride	2500	32	91	9	0	0	0
Eston Thinner	300	30	67	20	10	3	0
Eston Thinner	3000	39	87	8	5	0	0
Westvaco Thinner	300	34	64	24	6	6	0
Westvaco Thinner	3000	33	67	18	6	9	0
Stanisol	300	35	80	11	9	0	0
Stanisol	3000	34	82	9	3	6	0
Xylene	250	35	85	6	3	3	3
Xylene	2500	33	82	12	6	0	0
Chloropicrin	500	34	76	18	3	3	0
Chloropicrin	5000	32	82	9	9	0	0
Carbon tetrachloride	60	37	76	8	8	5	3
Carbon tetrachloride	200	37	64	22	8	3	3

---

[a] An unknown check was included in each test. Taste panel for each test was selected from those available of a group of about 25 persons, hence panels for all tests did not consist of the same individuals.

[b] Calculated from combined data of three separate tests on each sample, except for samples treated with 3000 pounds of Stanisol, which were examined in two tests only.

Table 2. Summary of taste tests on carrots grown in 1950 in plots treated in 1949 with various soil fumigants and other chemicals, each at dosages recommended for annual applications and at single dosages based on amounts normally applied in 10 years.

Material	Dosage (lb/acre)	No. of opinions[a]	% of opinions[b] rating samples as indicated off-flavor: None	Weak	Moderate	Strong	Objectionable
None (unknown check)	---	255	75	16	4	3	2
D-D mixture	250	37	32	19	14	11	24
D-D mixture	500	40	52	20	13	10	5
D-D mixture	5000	44	30	25	20	11	14
Dowfume N	250	42	57	21	10	7	5
Dowfume N	500	37	59	16	11	11	3
Dowfume N	5000	33	52	18	18	6	6
Ethylene dibromide	25	37	43	22	22	5	8
Ethylene dibromide	50	41	38	15	15	20	12
Ethylene dibromide	500	41	58	27	10	5	0
Dichlorobutenes	250	35	54	20	14	6	6
Dichlorobutenes	500	44	30	18	18	7	27
Dichlorobutenes	5000	-	-	-	-	-	-
Propylene dichloride	250	34	58	12	18	9	3
Propylene dichloride	2500	40	44	15	10	18	13
Eston Thinner	300	35	50	9	23	9	9
Eston Thinner	3000	42	52	14	19	10	5
Westvaco Thinner	300	35	63	20	11	6	0
Westvaco Thinner	3000	43	38	21	23	9	9
Stanisol	300	39	56	18	8	13	5
Stanisol	3000	22	63	5	14	9	9
Xylene	250	42	41	31	12	14	2
Xylene	2500	36	47	17	22	3	11
Chloropicrin	500	44	44	18	25	11	2
Chloropicrin	5000	38	32	21	21	13	13
Carbon tetrachloride	60	37	48	27	19	3	3
Carbon tetrachloride	200	41	54	12	20	12	2

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[a] An unknown check was included in each test. Taste panel for each test was selected from those available of a group of about 25 persons, hence panels for all tests did not consist of the same individuals.

[b] Calculated from combined data of three separate tests on each sample, except for samples treated with 3000 pounds of Stanisol, which were examined in two tests only.

Table 3. Yields of carrots and potatoes on chemical residue plots, Riverhead, Long Island, 1950, 1951, 1952.

Treatment	Dosage (lb/acre)	Years applied	Yields[a] - Bushels per acre of U. S. no. 1 carrots 1950	carrots 1951	carrots 1952	potatoes 1950	potatoes 1951	potatoes 1952
Check	---		567	816	720	583	420	222
D-D mixture	250	1949, 1950, 1951	537	841	795	565	546°°	279
D-D mixture	500	1949, 1950, 1951	607	866	698	562	477°	231
D-D mixture	5000	1949	449	802	666	396**	444	278
Propylene dichloride	250	1949, 1950	505	813	688	488*	459	257
Propylene dichloride	2500	1949	604	804	709	542	483°	303
Eston Thinner	300	1949, 1950	623	866	795	550	427	281
Eston Thinner	3000	1949	628	834	742	527	434	249
Westvaco Thinner	300	1949, 1950	604	849	795	542	453	319
Westvaco Thinner	3000	1949	529	852	677	542	429	269
Dowfume N	250	1949, 1950, 1951	720	859	742	580	470°	329
Dowfume N	500	1949, 1950, 1951	588	917°	784	558	471°	250
Dowfume N	5000	1949	432*	788	688	311**	455	310
Ethylene dibromide	25	1949, 1950, 1951	505	764	610	494	425	293
Ethylene dibromide	50	1949, 1950, 1951	677	806	645	553	495°	306
Ethylene dibromide	500	1949	653	780	720	535	430	322
Stanisol	300	1949, 1950	510	834	763	545	433	312
Stanisol	3000	1949	591	770	698	521	480°	238
Xylene	250	1949, 1950	607	852	731	532	455	285
Xylene	2500	1949	672	759	688	518	505°	262
Chloropicrin	500	1949, 1950, 1951	596	891°	677	599	480°	342
Chloropicrin	5000	1949	594	770	763	641	508°	313
Dichlorobutenes	250	1949, 1950, 1951	596	856	688	597	481°	315
Dichlorobutenes	500	1949, 1950, 1951	623	951°°	623	606	451	266
Dichlorobutenes	5000	1949	126**	838	698	0**	536°°	310
Carbon tetrachloride	60	1949, 1950	578	813	655	504	416	292
Carbon tetrachloride	200	1949	545	917°	827	524	473°	275

[a]Weight of tops included in yields for 1951, 1952, not in 1950.

LSD at 19:1			150	66		93	43	
LSD at 99:1			199	104		123	68	

° Increase over check at 19:1
°° Increase over check at 99:1
* Decrease from check at 19:1
** Decrease from check at 99:1

Table 4. Summary of taste tests on potatoes grown in 1951 in plots treated in 1949 and 1950 with various soil fumigants and other chemicals, each at dosages recommended for annual applications and at single dosages based on amounts normally applied in 10 years.

Material	Dosage (lb/acre)	No. of opinions[a]	% of opinions[b] rating samples as indicated off-flavor: None	Weak	Moderate	Strong	Objectionable
None (unknown check)	---	84	82	8	6	2	2
None (known check)	---	84	97	1	2	0	0
D-D mixture	500	36	75	19	0	6	0
D-D mixture	5000	35	85	9	6	0	0
Dowfume N	500	35	82	9	3	6	0
Dowfume N	5000	34	82	12	6	0	0
Ethylene dibromide	50	36	71	14	6	3	6
Ethylene dibromide	500	36	77	17	3	3	0
Dichlorobutenes	250	36	83	8	6	0	3
Dichlorobutenes	500	38	68	13	13	3	3
Dichlorobutenes	5000	24	75	13	8	0	4
Chloropicrin	5000	36	66	22	6	3	3

[a]An unknown check was included in each test. Taste panel for each test was selected from those available of a group of about 25 persons, hence panels for all tests did not consist of the same individuals.

[b]Calculated from combined data of three separate tests on each sample.

Table 5. Summary of taste tests on carrots grown in 1951 in plots treated in 1949 and 1950 with various soil fumigants and other chemicals, each at dosages recommended for annual applications and at single dosages based on amounts normally applied in 10 years.

Material	Dosage (lb/acre)	No. of opinions[a]	% of opinions[b] rating samples as indicated off-flavor: None	Weak	Moderate	Strong	Objectionable
None (unknown check)	---	102	58	15	10	12	5
None (known check)	---	102	88	8	1	1	2
D-D mixture	500	45	44	16	20	11	9
D-D mixture	5000	44	30	25	16	20	9
Dowfume N	500	44	50	16	14	11	9
Dowfume N	5000	42	38	24	14	7	17
Ethylene dibromide	50	47	36	28	21	9	6
Ethylene dibromide	500	43	44	14	28	12	2
Dichlorobutenes	250	43	42	23	7	14	14
Dichlorobutenes	500	41	36	17	15	15	17
Dichlorobutenes	5000	30	33	23	17	17	10
Chloropicrin	5000	43	68	16	12	2	2

[a]An unknown check was included in each test. Taste panel for each test was selected from those available of a group of about 25 persons, hence panels for all tests did not consist of the same individuals.

[b]Calculated from combined data of three separate tests on each sample.

Table 6. Composition of commercial materials applied to residue plots.

Material	Composition
Chloropicrin	trichloronitromethane
D-D mixture	1,3-dichloropropene; 1,2-dichloropropane
Dichlorobutenes	1,4-dichloro-2-butene
Dowfume N	1,3-dichloropropene; 1,2-dichloropropane
Eston Thinner	Petroleum thinner
Ethylene dibromide	1,2-dibromoethane
Propylene dichloride	1,2-dichloropropane
Stanisol	Petroleum thinner
Westvaco Thinner	Petroleum thinner

made. The cauliflower plants were dug with a potato digger and the roots scored for club-root. During harvest random samples of carrots and potatoes were removed from each plot for the taste determinations.

The taste tests were run by cooking approximately 2 pounds as a sample. Both carrots and potatoes were peeled. The carrots were sliced and the potatoes sectioned for cooking. An equal amount of water and salt was added to each sample before cooking and the samples were cooked for a given length of time. The carrots were served sliced and the potatoes were mashed. The judges served themselves from the cooking pots -- any amount desired -- to numbered paper cups. Tests on the carrots were run separately from the potatoes. Five treatments with a check and known check, for comparison, were included in each test. Samples, except for the known check, were listed only by number. At least 10 judges made comparisons for each test. Thus there were at least 30 opinions in the three replications for each treatment. Judges were asked to record their opinion for each sample on a printed form as to the off-flavor, if any, as regards taste and odor. If they judged the sample to be off-flavor, compared with the standard known check, they were to classify it as objectionable, strong, moderate, or weak.

RESULTS

Yields: Both carrot and potato yields were reduced significantly the first year (Table 3) with an application of 5000 pounds of D-D, Dowfume N and dichlorobutenes. No potatoes emerged on the plots treated with 5000 pounds of dichlorobutenes and the carrots were stunted and roots highly pronged. Consequently, samples of potatoes and carrots from this treatment were not available for taste tests the first year.

Off-flavor: The results (Tables 1, 2, 4, and 5) are shown as the total number of opinions in each designated flavor class in the three replications over the total judgments given. The most consistent data were obtained from the potato samples. Wide variability was evident in the carrot samples. The only off-flavoring consistently reported was in D-D and Dowfume N at the 10-year dosage of 5000 pounds. The annual treatment of 500 pounds of dichlorobutenes resulted in significant off-flavor in carrots but not in potatoes.

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TREATMENTS FOR ERADICATION OF DITYLENCHUS DIPSACI IN CLOVES OF GARLIC

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Summary

Eradication of Ditylenchus dipsaci from loose cloves of garlic was achieved without injury to cloves in greenhouse and laboratory tests. The treatment consisted of a pre-soak at 75° F in water containing 0.5% formalin and 0.1% detergent, followed by exposure to hot water at 120° for 20 minutes in a solution of the same composition as that for the pre-soak. No chemical dip or soak tested was effective without injury to the cloves. Examination of cloves showed nematodes present only in the surface tissues, including the thin protective leaves and abscission zone where the clove is attached to the stem. None were found in the storage leaf tissues.

The race of the stem and bulb nematode, Ditylenchus dipsaci, that attacks garlic and onion may be carried on bulbs and seeds. It has been eradicated successfully from onion seed by fumigation with methyl bromide (4). Garlic produces no true seed and propagation is accomplished by planting sections of the bulb called cloves. Much of the garlic grown for propagation is infested with D. dipsaci, which often causes severe losses. In addition, new areas become infested by planting infested cloves, and subsequent plantings of garlic and onions on such fields have resulted in near crop failures. With a limited supply of nematode-free garlic available for planting, there is need for an effective treatment to eliminate stem and bulb nematode from cloves of garlic used for propagation. The following experiments were conducted over a period of several years in attempts to use chemical dips or soaks, hot water, and combinations of both for this purpose.

METHODS AND RESULTS

Cloves of California Late garlic infested with Ditylenchus dipsaci were dissected to determine the location of the nematodes within the clove. Mann (5) has described the anatomy of the garlic bulb; each bulb contains several segments called cloves. Cloves used for planting are obtained by "cracking" or breaking the cloves loose from the basal plate of the dried bulb. The storage leaf constitutes the bulk of the weight and volume of the clove (Fig. 1) and is enclosed in a thin protective leaf which is closely appressed to it. The abscission zone is at the lower end where the clove is attached to the stem.

Protective leaves were removed from cloves and stained with acid fuchsin-lactophenol. Storage leaves were sectioned and stained in the same manner. Basal portions of cloves, including the abscission zone, were dissected and examined for nematodes. Nematodes were found only in the protective leaf and the abscission zone (Fig. 2), none within the tissues of the storage leaf. As an additional check on location of nematodes, protective leaves were removed from cloves and the abscission zone carefully removed. The storage leaves were thoroughly washed and some were planted and others were chopped into Baermann funnels. No nematodes were recovered from the funnels or from garlic

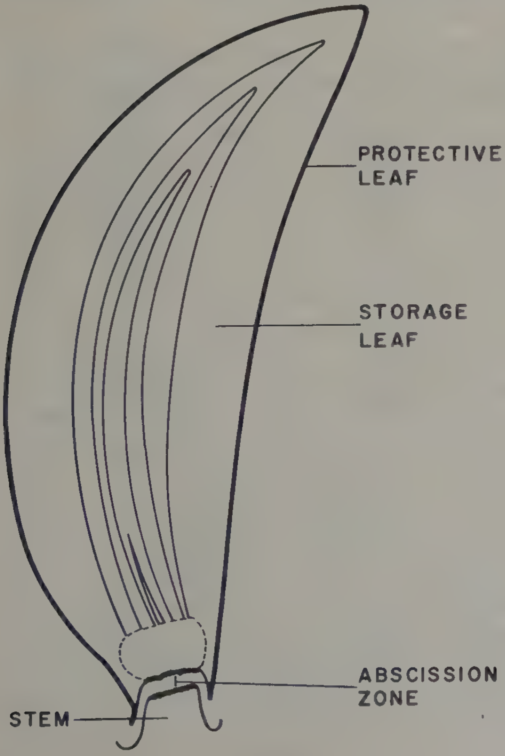

FIGURE 1. Diagram of garlic clove.

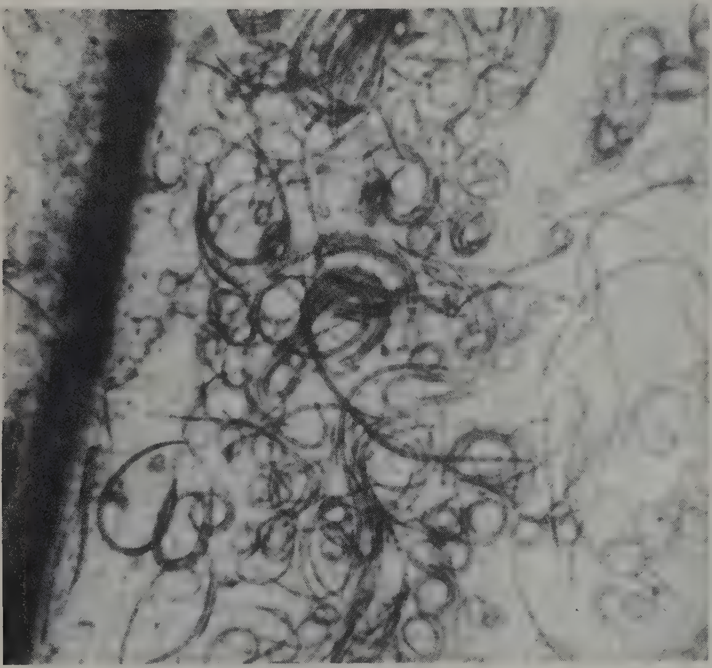

FIGURE 2. Photomicrograph of section of protective leaf of garlic clove showing position of Ditylenchus dipsaci. In the process of staining, many of the nematodes relax and assume an extended form. Normally, dormant fourth-stage larvae are coiled and closely packed within the leaf tissue.

Table 1. Chemical treatments used on garlic cloves for the control of Ditylenchus dipsaci.

Chemical	Concentration	Treatment time (minutes)	Viable nematodes recovered
TAF	2% triethanolamine; 7% formalin; 91% water	60	Yes
MAF	2% monoethanolamine; 7% formalin; 91% water	60	Yes
Propargyl alcohol + 2% triethanolamine	10, 000 ppm (v/v)	120	Yes
Propargyl alcohol	500 ppm (v/v)	120	Yes
Polyvinylpyrrolidone-iodine	2. 0% (w/w)	120	Yes
1,3-dichloropropene (95%)	500 ppm (w/w)	20	Yes
Phenol	0. 5% (v/v)	30	Yes
Sulfuric acid (cloves not pre-soaked)	36N	4	Yes
Sulfuric acid (cloves pre-soaked)	36N	4	Yes
Methyl bromide	2 lb/1000 ft^3	120	Yes
Sodium n-methyl dithiocarbamate	10, 000 ppm	48 hours	Yes
O-2,4-dichlorophenyl O,O-diethylphosphorothioate	2%	60	Yes
O,O-diethyl S-(ethylthiomethyl) phosphorodithioate	0. 96%	60	Yes
2,4,5-trichlorophenol, sodium salt	5%	60	Yes
	10%	60	No[a]

[a] Cloves killed by this treatment.

Table 2. Relative efficacies of hot-water treatment for elimination of Ditylenchus dipsaci from garlic cloves.

Treatment		Recovery of larvae from tissues placed in Baermann funnels			% plant stand 90 days after planting cloves
Temp. ° F	Time (minutes)	fresh cloves[a]	dried cloves[b]	leaves[c]	
110	120	0	0	+	91
118	20	+	0	+	71
118	25	0	0	0	87
120	15	0	0	+	84
120	20	0	0	0	87
120	25	0	0	0	90
122	15	0	0	0	82
Check	--	+	+	+	38

[a] Cloves placed on funnels immediately following treatment.
[b] Cloves dried for 3 months following treatment.
[c] Basal leaves from plants emerging from treated cloves.

Table 3. Number of larvae recovered from garlic cloves exposed to treatment with hot water with and without soaking prior to treatment.

Treatment intervals (minutes)		No. larvae recovered/clove
pre-soak[a]	120° F[b]	
0	0	1800 ± 700
150	0	450 ± 100
0	20	25 ± 14
150	20	0

[a] Solution containing 1% formalin and 0.1% detergent at 75° F.
[b] Same solution as pre-soak.

plants grown from planted cloves. Because the nematodes are superficially located on cloves, their eradication would appear feasible without damage to the clove.

Chemical Treatments: Cloves of California Late garlic known to be infested with D. dipsaci were selected and replicate lots of 12 cloves were immersed in the desired solution or emulsion. Immediately after the exposure period, cloves were washed to remove residual chemical and spread to dry. Half of the number of cloves in each replicate were planted to determine germination and growth characteristics. The remaining cloves were utilized to assay nematode control by placing the basal portion and protective leaf tissues on Baermann funnels. The methyl bromide treatment was conducted in a space fumigation chamber. Table 1 lists only the highest concentration-time exposure tested. None of these treatments was successful in eliminating the nematode without injury to cloves.

Dry Heat Treatments: Replicate lots of cloves of California Late garlic were placed in an oven maintained at 110° F for 24 and 48 hours; 120° for 16 and 24 hours; and 130° for 8 hours. Nematode assays and germination studies were determined as under the chemical treatments. Only at a temperature of 130° for 8 hours were no nematodes recovered. At this temperature, however, only 38% germination of cloves was obtained. In the other treatments, germination of cloves varied from 70% at 120° for 24 hours to 100% at 110° for 48 hours. An additional test used radio high-frequency waves as a heat source. The equipment was furnished and operated by F. C. Jacob of the Department of Agricultural Engineering. A 2-minute exposure to cloves resulted in temperatures as high as 165° in the center of the clove as measured by a thermocouple insert immediately following treatment. Viable nematodes were recovered from all treated cloves and germination of planted cloves was only 6%.

Hot-Water Treatments: The thermal death point of D. dipsaci in vitro was determined in order to establish a range of suitable temperatures. The procedure was to add 0.5 ml of a suspension of fourth-stage larvae to 5 ml of water at the desired temperature maintained in a water bath. At the end of the desired exposure period the suspension was rapidly transferred to approximately 100 ml of water at a temperature of 68° to 70° F, effecting a rapid cooling of the suspension. The suspensions were inspected after 48 hours and those in which active larvae were present discarded. Untreated suspensions were carried in the experiment as checks.

Results indicate that temperature-time combinations greater than 120° F for 16 minutes and 125° for 4 minutes are lethal to the nematodes.

Further investigation determined effect of the various temperatures and times of treatment on the germination and growth habit of the clove. Also included was study of the effect of pre-soaking cloves in conjunction with a hot-water treatment.

Results of these experiments indicate that garlic cloves tolerate a temperature of 122° F for 20 minutes and 120° for as long as 25 minutes. Exposure to temperatures of 124° and higher resulted in injury to the cloves.

Wetting garlic scales tends to activate dormant larvae which should increase their susceptibility to subsequent treatment. Formalin has been used as an additive to hot-water baths for the control of this nematode on other bulbs (1, 2). Treatments combining both pre-soaking and heat did not injure garlic cloves and viable nematodes were not recovered from cloves treated for 20 minutes at 120° F.

To verify preliminary results, the following experiment was conducted. Garlic bulbs known to be infested were cracked and the larger cloves, those most suitable for planting, were selected for treatment. Approximately 9 pounds of cloves were mixed to reduce differences due to variation in infestation. One-pound lots of cloves were pre-soaked in a water solution of 1% formalin containing 0.1% detergent for 150 minutes. Following this, the cloves were transferred to a wire basket and immersed in a treatment solution of the same composition as the pre-soak solution. To insure movement of the solution in the mass of cloves, the basket was agitated frequently for the first 2 minutes. At the end of the treatment interval, some of the cloves were removed and planted without cooling or rinsing. A sample of 10 cloves was taken from each treatment to assay nematode control. The balance of the cloves were dried and stored for 3 months, after which attempts were made to extract living nematodes. Other cloves were planted to determine the effect of drying on clove germination. Control of nematodes was based on numbers of larvae emerging from Baermann funnels containing chopped sections of treated cloves and from bulbs and foliage leaves of plants growing from treated cloves. In two treatments nematodes were recovered only from the foliage leaves and not from the treated cloves. The assay of outer foliage leaves for presence of nematodes has proved very useful since samples may be taken without destruction of the garlic plant. Foliage leaves infested with this nematode usually show abnormal thickening and yellowing with age. Godfrey and Scott (3) noted the presence of many nematodes in lower yellow foliage leaves but noted no distortion.

Table 2 indicates that a treatment at 120° F for 20 or 25 minutes resulted in nematode control and had no harmful effect on the cloves. At the higher temperature of 122° an exposure of 10 minutes provided nematode control, but this temperature approaches that where cloves are damaged.

On the basis of these results, the temperature-time combination of 120° F for 20 minutes was selected for further investigation. The entire treatment was broken into its component parts which were tested separately. The results (Table 3) indicate the necessity for the combined treatment. The pre-soaking treatment resulted in the elimination of approximately 75% of the nematodes in the cloves. The heat treatment applied separately resulted in a reduction of nematodes of approximately 99%. A combination of both pre-soaking and heat resulted in eradication.

DISCUSSION

From the standpoint of economics, a dip treatment at ambient temperatures would be desirable. Such a treatment would be dependent upon the ability of the toxicant to penetrate garlic tissues and retain its nematocidal activity. None of the chemicals used was successful without injury to cloves, either in germination or on growth characteristics of the resulting garlic plant. The resistance of the nematode to chemicals was emphasized by its survival in sulfuric acid. This treatment charred protective leaf tissues from which viable nematodes were later recovered.

Dry heat-type treatments have not proved satisfactory in the laboratory. The inefficiency of dry heat in controlling this nematode has also been observed in the field. Active D. dipsaci have been recovered from plant material obtained from the soil surface of garlic fields in Imperial County, California during the latter part of the summer. The garlic crop had been harvested in the May or June preceding. In this area temperatures at the soil surface may reach 156° F for short periods of time during the summer.

Moist heat has been more effective in controlling nematodes. Hot-water treatments for bulbs and root materials are effective for control of certain nematodes. The treatment of 110° F for 3 to 4 hours as recommended for control of stem and bulb nematode on narcissus and certain other bulbs was not successful in our studies with garlic cloves. Inconsistent results on nematode control and clove injury were obtained in numerous tests. Clove tolerance appears to be associated with degree of dormancy. Cloves treated near time of harvest tolerated heat better than those treated at planting time. Complete eradication of the nematode was achieved in only a limited number of tests. Similar results were obtained with a treatment of 115° for 2 hours, a treatment which has had some commercial use.

The treatment found effective in our studies is useful only for the treatment of loose cloves and not for intact bulbs. It should be emphasized that the data presented are based on greenhouse and laboratory studies in which quantities of cloves treated were at most 1-pound lots. For field use, of course, large quantities of cloves must be treated if the treatment is to be of commercial value. Most of these experiments used late garlic; early garlic was used to a limited degree. The Creole variety has not been treated and its tolerance to this treatment is unknown. The largest obstacle to the commercial use of this treatment probably will be availability of hot-water tanks with adequate temperature regulating devices and means of agitation. It appears to the authors, however, that the materials required are readily available and the treatment time sufficiently brief that the method should find application.

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CYNEM -- AN EFFECTIVE CONTROL FOR STING NEMATODES ON PEANUTS

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Summary

Surface applications of a 10% granular formulation of O,O-diethyl O-2-pyrazinyl phosphorothioate (Cynem) at 1, 2, 3, 4 and 6 pounds active per acre to the row at time of planting resulted in peanut yields of 1948, 2363, 2387, 2283 and 2604 pounds/acre, respectively, compared with 1026 pounds/acre for the nontreated control. Acre values for the respective yields were $218, $289, $293, $284, $329 and $111, respectively. Liquid applications of 2, 4 and 6 pounds/acre were slightly less effective than the comparable granular applications. Preharvest ratings of the effect of Cynem treatments on plant size were directly correlated with yields and acre values, whereas pod and root ratings for symptoms of nematode injury were inversely correlated.

INTRODUCTION

For the past 4 years considerable emphasis has been placed on chemical control of the sting nematode, Belonolaimus longicaudatus Rau, in North Carolina. This was the result of an increasing recognition of the damage caused by this nematode to peanuts and of the lack of other adequate control measures. For many plant-parasitic nematode species some measure of control can be obtained by the use of resistant crops in the rotation. In the peanut production area of North Carolina, however, crops normally grown in rotation with peanuts -- namely, corn, cotton and soybeans -- are also highly susceptible to sting nematode injury. Furthermore, growth of these crops greatly increases the population of the sting nematode and the possibility of a crop failure if peanuts are planted in these fields without some other means of control.

Investigations conducted in North Carolina in 1958 and 1959 (1, 4) evaluated 1,2-dibromo-3-chloropropane (DBCP) as to effective rates and relative efficacy of preplant, at planting, and postplant applications. In Virginia (2) over a period of several years DBCP, D-D and EDB were tested extensively as fumigants for control of sting nematode on peanut. These nematocides are used on considerable peanut acreage in North Carolina and Virginia.

In 1958, 1959 and 1960, Cynem was evaluated for nematode control at dosages from 4 to 16 pounds/acre of active chemical using a 10% granular formulation (3, 4). Both row and broadcast treatments were tested. Results indicated that control, as reflected by peanut yield and acre value, was effective at 4 pounds/acre. These studies, however, failed to indicate the lower limits of effectiveness. To determine this the 1961 test was designed to evaluate Cynem at lower dosages and to compare the effectiveness of liquid and granular formulations.

MATERIALS AND METHODS

Soil Preparation: The debris of the preceding peanut crop was buried during March by turning the soil. The field was disked and harrowed just prior to application of the treatments and the planting operation.

Plot Design and Treatments: The experimental design was a randomized block of two-row (6 x 130 foot) plots with four replicates. Applications were to the soil surface in a 12-inch band immediately in front of the planter during planting operation on May 28. A 10% granular formulation was applied at 0, 1, 2, 3, 4, and 6 pounds active per acre with a Gandy distributor. An emulsifiable concentrate (4 pounds active per gallon) was diluted with water so that 10 gallons/acre of the mixture gave 2, 4, and 6 pounds active per acre and applied with a sprayer at 30 psi using flat spray nozzles. Planting and treatment operations were simultaneous. The only mixing of the nematocide into the soil was that incident with the planting operation. Subsequent cultural practices were essentially those recommended by the North Carolina Agricultural Extension Service for peanut production in this area.

Determination of Nematode Population: An appropriate number of soil plugs 6 to 8 inches deep were taken from the root zone of plants in each plot with a 1-inch sampling tube on July 13. The nematodes were recovered from the soil by a modified Baermann funnel technique. Counts were made under a dissecting microscope (36X).

Preharvest Ratings of Plant Size and of Pod and Root Symptoms of Sting Nematode Injury:

Table 1. A comparison of Cynem treatments of sting nematode-infested soil on sting nematode populations, plant size, disease control, yield, grade, and acre value of peanuts - 1961.

Cynem formulation (pounds/acre)		Nemas/pint soil[a]	Sting nematode symptoms on: plant size[b]	roots[b]	pods[b]	Yield (pounds/acre)	Grade (cents/pound)	Value (dollars/acre)
Nontreated	0	75	1.12	1.00	1.25	1026	10.80	$111
Granular	1	50	3.25	3.00	3.25	1948**	11.18	218
Granular	2	25	4.38	3.54	3.71	2363**	12.25	289
Liquid	2	25	3.19	3.54	3.21	1913**	11.01	211
Granular	3	38	4.12	3.83	3.54	2387**	12.26	293
Granular	4	6	3.56	4.04	3.62	2283**	12.42	284
Liquid	4	56	3.62	3.62	3.42	2185**	12.51	273
Granular	6	31	4.62	4.25	4.04	2604**	12.64	329
Liquid	6	13	4.31	3.96	3.96	2384**	12.34	294
Correlation with yield (r)		--	0.974	0.961	0.975	----	-----	---
Regression of yield on (b)		--	435	453	552	----	-----	---

[a] Estimated number of sting nematodes per pint of soil based upon Baermann funnel technique, 7/13/61.

[b] The ratings were from 1 (for most severely stunted plants or most severe symptoms) to 5 (for normal plants or no sting nematode symptoms), 9/19/61.

**Significant at .01 level.

To evaluate the degree of control as indicated by stunting of plants and symptoms of sting nematode injury to roots and to pods, each plot was rated from 1 (severely stunted plants or most severe symptoms) to 5 (normal plants or no sting nematode symptoms) for each category on August 19. Plant size was rated independently by four individuals and was based upon the average condition of the entire plot. Symptoms of nematode injury on roots and on pods were rated independently by three individuals and was based upon the average condition of two hills, one from each row per plot, taken in line across the entire test.

Harvest Operations: The peanut plants from each plot were removed on October 19 and stacked on a pole in the field. After drying until November 29, they were picked with a conventional, cylinder type, peanut picker. The harvested nuts from each plot were cleaned to remove the larger stem fragments before weighing. Samples of nuts for chemical residue analysis and market grade evaluation for each treatment were obtained by pooling a given quantity from each plot which received the same treatment. Market grade evaluations were made by the U. S. Marketing Service personnel.

EXPERIMENTAL RESULTS

Preharvest Evaluations of Cynem Treatments: The preharvest ratings by the individuals for plant size and for symptoms of nematode injury to pods and roots showed close agreement. The average preharvest ratings for each category of symptoms are shown in Table 1. In all three categories the greatest increase was between the untreated control and the 1 pound/acre rate. There was, however, a further increase with the 2-pound rate over the 1 pound. Rates higher than 2 pounds/acre were not appreciably more effective than 2 pounds/acre. In general the granular formulations were more effective than the liquid. All chemical treatments reduced the population of sting nematode below that of the check.

Very high correlations exist between the preharvest ratings for plant size and symptoms of pod and root injury and yields. There is also a close correlation between the ratings for the three categories. The regression values for all three categories are indicative of this.

Yields, grades and acre values for pods from the various treatments are shown in Table 1. The greatest increase in yield and acre value per pound of nematocide over that of the untreated control was with the 1 pound/acre rate. The 2 pound/acre rate granular increased the yield and acre values over that of the 1-pound rate, but higher dosages did not result in consistent increases.

A comparison of the effectiveness of given dosage levels over a 3-year period is presented in Table 2. As indicated earlier, the greatest yield increase per unit nematocide in the 1961 test is between the nontreated plots and those treated at the 1 pound/acre rate. Although with

Table 2. Effect of Cynem treatments of sting nematode-infested soil at planting in 1959, 1960, and 1961 upon peanut yield.

Year	Average yield of peanuts (pounds/acre) from plots treated with Cynem at indicated rates (pounds/acre)							
	0	1	2	3	4	6	8	16
1959	1048	----	----	----	1947	----	2451	3013
1960	787	----	----	----	2275	----	2554	2816
1961	1026	1948	2363	2387	2383	2604	----	----

dosages from 2 to 6 pounds/acre yields were greater than at 1 pound/acre there was no significant increase with more than 2 pounds/acre. In the earlier tests, however, the 8 and 16 pound /acre rates resulted in significant yield increases above the 4-pound rate. These yield increases were directly correlated with increased dosage, indicating that the upper limits of effectiveness as expressed by yield had not been attained.

DISCUSSION AND CONCLUSION

Data presented here and those given in previous reports (3, 4) indicate the effectiveness of Cynem in increasing yields, quality and acre values of peanuts when applied to sting nematode-infested soil. The consistent results with this material over the 3-year period is of interest, since growing seasons, nematode infestations, and other ecological factors vary from year to year.

While 1 pound/acre gave significant yield increases over that of nontreated plots, the use of 2 pounds/acre, especially in highly infested fields, would result in further economical increases in yield and quality. In earlier tests the yield increases with 8 and 16 pounds/acre over the 4-pound rate may indicate more nearly the actual damage caused by this nematode as well as the low level of phytotoxicity of the nematocide.

These studies further indicate that the sting nematode is responsible for reduced plant size and for the development of characteristic symptoms on peanut roots and pods. The intensity of these symptoms is highly correlated with yields.

The reason why the granular formulation was generally superior to the liquid formulation is not known.

Of the various nematocides available today, Cynem appears to be the simplest to apply with conventional equipment and labor. It can be effectively applied to the top of the row during the planting operation, therefore it does not require a waiting period before planting to pull buried debris of the preceding crop to the surface with deeply set application equipment. Either granular or liquid formulations can be metered with more or less conventional devices. Broad usage of this nematocide, however, will depend upon its effectiveness on a wide range of crops and nematode species.

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PATHOGENICITY OF PHYTOPHTHORA PALMIVORA IN THE ORCHIDACEAE[1]

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Summary

Plants in the following genera in the Orchidaceae were susceptible to wound inoculation with zoospores or mycelium of Phytophthora palmivora: Cattleya, Dendrobium, Epidendrum, Paphiopedilum, and Vanda. Plants in the genera Angraecum, Phalaenopsis, Spathoglottis, and Vanilla were resistant. Only plants in the genus Vanda or Epidendrum became diseased when non-wounded mature plants were inoculated with zoospores. Optimum temperature for zoospore production was between 15° and 25° C with none produced at 28°. Sporangia and chlamydospores were produced on the host between 20° and 31°. Leaf and stem infection with zoospores on wounded Vanda leaves occurred at temperatures from 15° to 31°.

INTRODUCTION

Thompson (6) adequately described the symptoms of Phytophthora palmivora (Butl.) on seedling hybrids and mature plants of Vanda orchid in Singapore. The pathogen was first described as a parasite of the genus Dendrobium in Ceylon in 1924 (3). Schwarz (5) recorded P. omnivora De By. on Vanda coerulea, Cattleya sp., Phalaenopsis amabolis, and Grammatophyllum scriptum in Java. The fungus was later identified by Ashby (1) as P. palmivora. Ela (2) described the disease from the Philippines and Gilbert, et al. (4) described heat treatments to eliminate a Phytophthora sp. from Cattleya in Hawaii, which presumably was the same disease.

The object of this study was to describe the symptoms of the disease under Hawaiian conditions, the host range of the pathogen in the Orchidaceae, and the role of temperature in dissemination, infection, and disease development.

MATERIALS AND METHODS

The following members of the Orchidaceae were tested for susceptibility to the fungus: Angraecum veitchii, Cattleya sp., Paphiopedilum sp., Dendrobium Caesar, Dendrobium Molokai, Epidendrum sp., Phalaenopsis sp., Spathoglottis plicata, Vanda Elinoa, Vanda Michael Beaumont, Vanda Miss Joaquim, and Vanilla sp. Non-orchidaceous plants tested were Chrysalidocarpus lutescens and Carica papaya.

In vitro tests with roots, stems, and leaves were conducted by placing the above tissues in moist chambers. Zoospore suspensions or V-8 agar blocks containing mycelium were placed on wounded or non-wounded tissues. Mature plants were inoculated with zoospore suspensions or with agar containing the fungus. They were placed in moist chambers for 48 hours at temperatures fluctuating from 23° to 27° C. The specificity of the in vitro testing was checked by inoculating leaves of the above plants with several Phytophthora spp., other than P. palmivora, including P. citrophthora (R. E. Sm. & E. H. Sm.) Leonian, P. cactorum (Leb. & Cohn) Schroet, P. parasitica Dast., P. megasperma Drechs., and P. cryptogea Pethyb. & Laff. The inoculation was listed as positive if the fungus killed tissue at least 10 mm from the point of inoculation after 5 days and could be reisolated from the margin between the rotted and healthy tissue.

Temperature Studies: The influence of temperature on zoospore formation was determined by growing the fungus on a medium composed of 10% V-8 juice, 0.2% $CaCO_3$, and 2% agar. Discs of agar containing sporangia were then transferred to small glass dishes containing distilled water at 2°, 4°, 12°, 15°, 20°, 25°, 28°, 31°, 34°, 37°, and 40° C and observations

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Table 1. Influence of water temperature on zoospore formation in P. palmivora.

Temperature of water in °C	Number of sporangia/100 producing zoospores[a] after: 1 hour	2 hours	5 hours[b]
2	0	0	0
4	0	0	0
12	68	72	75
15	84	92	95
20	89	98	98
25	56	95	97
28	0	0	0

[a] Discs of V-8 medium with sporangia were transferred to water at the indicated temperatures.

[b] The percentages did not change after 5 hours. No zoospores were produced at 2°, 4°, 28°, 34°, 37°, and 40° C even after 24-hour incubation periods.

Table 2. Influence of water temperature on the length of time zoospores of P. palmivora remain active.

Temperature of water in °C	% encysted and germinated zoospores at:[a] 2 hours	5 hours
12	.12	66
15	63	84
20	62	85
25	0	37

[a] 0% = 100/100 zoospores still actively swarming.
37% = 37/100 zoospores have encysted and germinated.

made on sporangial germination. Discs of the fungus were transplanted on V-8 agar and daily measurements of mycelial growth noted at the different temperatures. Zoospores collected from sporangia germinated at 12°, 15°, 20°, and 25° were placed in water at these temperatures and duration of motility noted.

The influence of temperature on infections was studied by placing zoospores suspensions on wounded or non-wounded strap-leaved Vanda leaves and then incubating the leaves at different temperatures in moist chambers.

RESULTS

In Hawaii, the disease most commonly expresses itself as a leaf, heart, and stem rot of plants in the strap-leaved Vanda group. Plants in the following genera of Orchidaceae, however, were shown to be susceptible to P. palmivora on wound inoculation with zoospores or mycelium: Cattleya, Dendrobium, Epidendrum, Paphiopedilum, and Vanda. Plants in the genera Angraecum, Phalaenopsis, Spathoglottis, and Vanilla were resistant. Only plants in the genus Vanda or Epidendrum became diseased when non-wounded mature plants were sprayed with zoospores.

In Vanda, infection may take place at any location on the stem or in the heart of the plant. The fungus then moves systemically upward and downward in the stem, killing the stem and causing leaf fall. Infection may occur on the leaves and the fungus moves into the stem. A black rot of the leaves is common. If the weather turns dry after infection, the disease may be arrested and only the crown and two or three upper leaves are blighted. If wet weather persists after the original crown infection, then new infections are initiated at other locations on the leaves and at leaf axils. The source of inoculum for secondary infection is from sporangia produced on the leaves from the primary infection.

The disease in Hawaii is less common in Cattleya, but leaves and pseudobulbs may be affected with a black rapidly progressing rot under optimum conditions.

There was little difference between the susceptibility or resistance of roots, stems, and leaves. If the plant was susceptible, these portions were all equally invaded and killed by the fungus.

Of the several Phytophthora spp. studied, P. parasitica, from papaya, readily invaded and rotted wounded leaves and stems of Vanda and Cattleya. The fungus was pathogenic, however, only when introduced into wounded tissue.

Movement of the fungus in susceptible host tissue was primarily a function of temperature. The fungus moved most rapidly in Vanda stems, approximately 3 mm/day at 28° C. Movement was also rapid in stems of Epidendrum and in leaves and pseudobulbs of Cattleya. In mature pseudobulbs, the movement was significantly slower than in young, herbaceous tissue. The optimum temperature for mycelial growth in the host was 28°.

The minimum temperature for mycelial growth on V-8 agar was about 10° C, optimum 28° to 31°. The fungus grew at 35° but not at 37°.

On V-8 medium and on stems or leaves of Vanda, the fungus produced sporangia and chlamydospores at temperatures from 20° to 31° C after 72 hours.

Optimum water temperatures for production of zoospores were between 15° and 25° C. No zoospores were produced at 28° (Table 1). The zoospores remained active for the longest time in water at 25° (Table 2). Strap-leaved Vanda leaves were infected with zoospores on wounded upper or lower leaf surfaces at temperatures from 15° to 31°.

DISCUSSION

Inhibition of zoospore production at temperatures above 25° C explains why the disease is not found during the hotter months of the year even when adequate moisture is present.

The disease is more common in the strap-leaved Vanda group because 1) non-wounded tissue may be invaded, and 2) zoospores are more readily trapped by the host in leaf axils. Water may stand for long periods of time in these leaf axils, which facilitates infection.

Possibly P. parasitica from papaya could cause disease in Vanda and Cattleya, as zoospores of this fungus readily invaded and killed wounded plants in these genera. The symptom expression was identical with that incited by P. palmivora. The two fungi, however, were not completely cross inoculable, as zoospores of P. palmivora were non-pathogenic to non-wounded leaves or stems of papaya; whereas, zoospores of P. parasitica were infectious under the same conditions.

Plants in several orchid genera were shown to be susceptible to P. palmivora when the fungus was introduced into wounded tissue. However, since only Vanda and Cattleya have been found naturally diseased in the field, this susceptibility may be assumed to be of academic interest only.

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CONTROL OF APHANOMYCES ROOT ROT OF PEAS IN THE GREENHOUSE WITH METHIONINE AND NORLEUCINE

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Abstract

Effective control of Aphanomyces root rot of peas in the greenhouse was obtained with two or three drenches of methionine or norleucine (at 70 ppm each) applied within a few days before or after soil infestation with zoospores. Total concentrations higher than 210 ppm were phytotoxic to peas. Methionine was more phytotoxic than norleucine at equal concentrations. One drench at 30 ppm reduced root rot, but reduction was less pronounced than that at 70 ppm. Root rot was not reduced if more than 3 days elapsed from soil infestation to the first drench application. Methionine and norleucine at 140 ppm as side-dressings, with or without fertilizers, applied 2 to 3 days before or 1 day after soil infestation reduced root rot greatly. The effectiveness of the amino acids was increased by combining them with fertilizers. Applications of methionine or norleucine below the seed or at the same level as the seed at planting time were less effective than drenches and side-dressings. In soil "naturally" infested with the pathogen drenches or side-dressings with methionine or norleucine at 140 ppm effectively controlled root rot, but methionine was injurious to the plants.

Recent nutritional studies in liquid and sand cultures showed that Aphanomyces root rot of peas, caused by Aphanomyces euteiches Drechs., could be prevented by several amino compounds (6). Additional studies (7, 8) demonstrated that all compounds that prevented Aphanomyces root rot of peas possess methyl (CH_3) and amino (NH_2) groups in their molecules. Methionine and its sulfur-free analogue norleucine were very effective.

The present study was undertaken to evaluate the effectiveness of methionine and norleucine[2] alone and in combination with fertilizers for control of Aphanomyces root rot of peas in small-scale greenhouse tests.

MATERIALS AND METHODS

The soil used was a non-sterilized, Aphanomyces-free, Elsinboro sandy loam with pH 5.8 and a moisture-holding capacity (MHC) of 40%. "Naturally" infested soil was obtained by infesting portions of Elsinboro sandy loam with A. euteiches and continuously cropping with susceptible peas until a high level of soil infestation was attained. The soil was brought to 50% MHC and portions equivalent to 3.5 kg of oven-dry soil were placed in plastic-lined No. 10 tin cans provided with drainage. Ten surface-disinfected seeds of Alaska peas were planted in each can. The soil moisture level in the cans was maintained at 50% MHC until the soil was infested with zoospores.

Zoospore inoculum of A. euteiches was prepared by a method described by Schneider and Johnson (9). Zoospore concentrations for the various tests were determined with a hemacytometer and adjusted to provide about 1.5 million zoospores per can of soil. Immediately after soil infestation with zoospores, soil moisture was raised to 75% MHC and maintained at that level for 1 week. A temperature of 20° C was maintained before soil infestation. Immediately after infestation it was raised to 25° and maintained at that level until the end of the experiments.

The materials used were DL-methionine (feed grade) supplied by The Dow Chemical Co.; DL-norleucine; KCl, NH_4NO_3, and 10-10-10 fertilizer. Methionine and norleucine were applied to soil as drenches, side-dressings, or during planting (2 inches below the seed or with the seed) at concentrations calculated as ppm of the dry soil weight. For soil drenches the material for each soil portion was dissolved in 100 ml of water and poured on the soil surface before or after soil infestation with zoospores. Side-dressings and applications during planting were made dry to the soil surface and in the soil, respectively. In "naturally" infested soil drenches were applied 7, 11, and 13 days after planting and side-dressings 7 and 11 days after planting. Ammonium nitrate and 10-10-10 were used at rates to provide 50 ppm of additional N and KCl at a rate to provide 105 ppm of additional K to the soil. In naturally infested soil, 10-10-10 was mixed with the entire soil 1 week before planting.

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[1] Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

[2] Chemicals mentioned not yet registered for uses reported as of June 19, 1962.

Four replications were used throughout. Each replication consisted of one can planted with 10 seeds. Three cans of noninfested soil were used as controls for each treatment, and most of the experiments were performed twice.

Three weeks after soil infestation, plant height was measured and the aboveground parts rated for general appearance on an arbitrary scale of 0 (all plants dead) to ++++ (all plants healthy). Plant roots were removed from soil and the extent of root rot was recorded on the basis of an arbitrary scale of 0 (no visible root rot) to 4 (extensive water-soaking and discoloration with disintegration of infected tissue) suggested by Smith and Walker (10). A disease severity index from 0 (all roots apparently healthy) to 100 (all roots and epicotyl rotted) was calculated by multiplying the mean infection rating by 25.

RESULTS

Soil Drenches: The disease severity index and general plant appearance indicated that methionine or norleucine drenches (before or after soil infestation) applied at various rates to provide total concentrations up to 420 ppm reduced Aphanomyces root rot considerably (Table 1). Drenches with total concentrations higher than 210 ppm reduced root rot greatly, but were of little benefit because phytotoxicity symptoms developed on the plants. At equal concentrations methionine was more phytotoxic than norleucine. Toxicity symptoms included stunting of plants, green netting of leaves, chlorosis of the upper leaves, and brown spots on the roots. The name "methionosis" has been suggested to describe similar disorders of various kinds of plants caused by methionine (12). One or three methionine or norleucine drenches (70 ppm each) within a few days before or after soil infestation resulted in appreciable control of root rot and healthy aboveground plants (Table 1). Drenches at 30 ppm reduced root rot, but less than drenches did at 70 ppm.

The time of drench application in relation to planting time or soil infestation was very critical in determining the effectiveness of the two amino acids. In one experiment (Fig. 1) 70 ppm of norleucine were effective in reducing root rot when applied 1 and 5 or 1 and 9 days after soil infestation with zoospores, but completely ineffective when applied 5 and 9 days after infestation. In all drench experiments, the smaller the plants at time of drench application the more pronounced the plant injury.

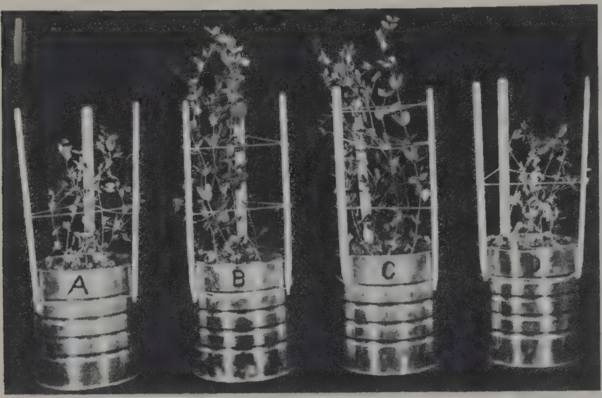
Side-dressings: Methionine or norleucine powders at 140 ppm added with or without fertilizers to the soil surface 2 days before zoospore addition reduced root rot severity greatly (Table 2). Plants grown in soil treated with methionine or norleucine with or without fertilizers had a better appearance and were taller than those grown in the control soil or in soil treated with fertilizers alone. All plants grown in the methionine-treated soil were shorter than those grown in the norleucine-treated soil. .

Disease severity indices with methionine side-dressings ranged from 10 to 18 and those with norleucine side-dressings from 3 to 25. Of all combinations tested 2 days before infestation, the best control and the best general plant appearance were obtained with norleucine + KCl and norleucine + 10-10-10. The three fertilizers used reduced disease severity to a small degree (disease severity indices with 10-10-10, KCl, and NH_4NO_3 were 68, 88, and 89, respectively) but were inferior to methionine or norleucine alone. All plants in the infested control soil were dead (Fig. 2).

Results obtained with side-dressings applied 1 day after soil infestation were similar to those with pre-inoculation side-dressings except that control was less pronounced (Table 2). Methionine was more effective than norleucine when the two amino acids were applied after soil infestation. With side-dressings applied after soil infestation, the best control was obtained with the combinations methionine + 10-10-10 or norleucine + 10-10-10.

A separate experiment was performed with side-dressings of methionine or norleucine at 35, 70, and 140 ppm 3 days before soil infestation with zoospores. At 140 ppm, disease severity indices of 3 for methionine and 18 for norleucine were observed; at 70 ppm indices for methionine and norleucine were 50 and 23, respectively; and at 35 ppm they were 68 and 55. In the control soil an index of 85 was observed. At 140 ppm methionine stunted the plants, but norleucine did not.

Applications Below the Seed or with the Seed: Methionine or norleucine powders at 140 ppm (with or without 10-10-10) applied 2 inches below the seed or at the same level as the seed during planting were less effective in reducing root rot than drenches or side-dressings (Table 3).



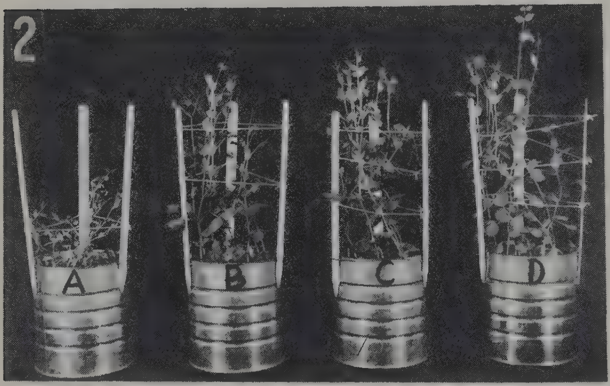


FIGURE 1. Peas in soil infested with A. euteiches as affected by norleucine drenches at 70 ppm. A -- no drench. B -- drench 1 and 5 days after soil infestation. C -- drench 1 and 9 days after soil infestation. D -- drench 5 and 9 days after soil infestation.

FIGURE 2. Peas as affected by norleucine side-dressings at 140 ppm applied 2 days before soil infestation with zoospores of A. euteiches. A -- none (infested control). B -- norleucine. C -- norleucine + NH_4NO_3. D -- norleucine + 10-10-10.

The 2-inch-below-the-seed application had only a slight depressing effect on disease severity. Applications at the seed level reduced root rot severity; reduction was more pronounced with methionine + 10-10-10 or norleucine + 10-10-10 than with either amino acid alone.

Soil Drenches, Side-dressings, and Applications with the Seed in "Naturally" Infested Soil: Methionine and norleucine applied as drenches, side-dressings, or at the same level as the seed during planting were very effective in reducing root rot of peas in soil "naturally" infested with A. euteiches (Table 4). Three methionine or norleucine drenches at 70 ppm each, one side-dressing with either amino acid at 140 ppm 7 or 11 days after planting, or two side-dressings at 70 ppm each were very effective in reducing root rot.

Plants side-dressed with methionine or norleucine had better appearance than those grown in soil that received drenches. Methionine drenches providing a total of 210 ppm and side-dressings providing 140 ppm of methionine in one dose stunted the plants and caused root injury manifested by small brown spots. Norleucine drenches providing a total of 210 ppm of norleucine caused slight root injury but not stunting. Norleucine side-dressings were not toxic at the concentrations used.

Applications of the amino acids with the seed at 140 ppm or at 70 ppm with the seed followed by a side-dressing at 70 ppm reduced root rot considerably (Table 4), but not so much as drenches or side-dressings. Applications with the seed reduced pea emergence to some extent, but did not injure the surviving plants.

DISCUSSION

Not very often do compounds effective against a disease-causing organism in test tubes prove effective against the same organism in natural soil. Methionine, norleucine, and a few other amino compounds possessing CH_3 groups in their molecules completely prevented root rot of peas caused by A. euteiches in liquid and sand cultures (6, 7, 8). The present greenhouse tests showed that methionine or norleucine drenches or side-dressings also satisfactorily controlled Aphanomyces root rot in soil. Although the two amino acids at relatively low rates were effective against this root disease, the high cost of these materials perhaps would not permit their use for practical control. Additional studies are needed to understand the behavior of these amino acids in field and greenhouse soils and to obtain a complete picture of the effects of the amino acids on peas and plants that follow peas and on soil microflora, especially pathogenic fungi of the pea root rot complex. Also, the effect of environmental factors such as temperature and soil moisture content on the effectiveness of amino compounds must be studied.

The time of treatment with respect to planting time and soil infestation with zoospores was of critical importance in determining the effectiveness of the two amino acids. Since the time of infection of peas grown in naturally infested field soil cannot be predicted, application of the amino acids with the seed during planting might seem the most expedient method of treatment. However, applications during planting were less effective than applications after pea emergence. When drenches or side-dressings were delayed 3 or more days after infestation, the amino ac-

Table 1. Root rot severity and appearance of peas as affected by methionine and norleucine soil drenches applied before and after soil infestation with zoospores of *Aphanomyces euteiches*.

Chemical and concentration (ppm)	Application day before (-) or after (+) soil infestation	Total concentration (ppm)	Disease severity index[a]	Plant appearance[b]
Methionine:				
30	+1	30	80	++
30	+1, +3, +6	90	68	++
30	-2, +1, +3	90	67	++
70	+1	70	65	+++
70	+1, +3, +6	210	38	+++
70	-2, +1, +3	210	25	+++
140	+1	140	53	++
140	+1, +3, +6	420	3	+
140	-2, +1, +3	420	35	0
Norleucine:				
30	+1	30	48	++
30	+1, +3, +6	90	43	++
30	-2, +1, +3	90	45	+++
70	+1	70	23	+++
70	+1, +3, +6	210	37	+++
70	-2, +1, +3	210	13	++++
140	+1	140	40	+++
140	+1, +3, +6	420	30	++
140	-2, +1, +3	420	2	+
None (infested control)	--	--	100	0

[a] Based on scale in which 0 indicates all roots apparently healthy and 100 all roots and epicotyl rotted.
[b] Symbols denote relative appearance of aboveground pea plants: 0 = all plants dead; ++++ = all plants healthy.

Table 4. Root rot severity and appearance of peas grown in soil "naturally" infested with *Aphanomyces euteiches* as affected by methionine and norleucine applications during or after planting.

Treatment and concentration		Application after planting (days)	Total concentration (ppm)	Disease severity index[a]	Plant appearance[b]
Methionine:					
Drench	(70 ppm)	7, 11, 13	210	5	+++
Side-dressing	(140 ppm)	7	140	0	+++
Side-dressing	(140 ppm)	11	140	3	+++
Side-dressing	(70 ppm)	7, 11	140	8	++++
With seed	(140 ppm)	0	140	28	++++
With seed and as side-dressing	(70 ppm)	0, 7	140	33	+++
Norleucine:					
Drench	(70 ppm)	7, 11, 13	210	0	++++
Side-dressing	(140 ppm)	7	140	23	++++
Side-dressing	(140 ppm)	11	140	15	++++
Side-dressing	(70 ppm)	7, 11	140	20	++++
With seed	(140 ppm)	0	140	45	++++
With seed and as side-dressing	(70 ppm)	0, 7	140	40	++++
None (control):					
With 10-10-10		--	---	63	++
Without 10-10-10		--	---	90	+

[a] Based on scale in which 0 indicates all roots apparently healthy and 100 all roots and epicotyl rotted.
[b] Symbols denote relative appearance of aboveground pea plants: 0 = all plants dead; ++++ = all plants healthy.

Table 2. Root rot severity, relative plant height, and appearance of peas as affected by methionine and norleucine side-dressings (with or without fertilizers) applied before or after soil infestation with zoospores of *Aphanomyces euteiches*.

Time and kind of treatment[a]	Disease severity index[b]	Plant height (% of non-infested in each treatment)	Plant appearance[c]
Two days before soil infestation:			
Methionine	18	93	+++
Methionine + KCl	15	88	+++
Methionine + NH_4NO_3	18	93	+++
Methionine + 10-10-10	10	94	+++
Norleucine	25	100	++++
Norleucine + KCl	8	100	++++
Norleucine + NH_4NO_3	23	93	++++
Norleucine + 10-10-10	3	100	++++
KCl	88	65	++
NH_4NO_3	89	51	0
10-10-10	68	84	++
None (infested control)	100	23	0
One day after soil infestation:			
Methionine	55	94	++++
Methionine + NH_4NO_3	50	98	++++
Methionine + 10-10-10	20	87	++++
Norleucine	60	88	+++
Norleucine + NH_4NO_3	58	86	+++
Norleucine + 10-10-10	35	92	++++
NH_4NO_3	80	77	+
10-10-10	90	78	+
None (infested control)	98	58	+

[a] The amino acids were added to soil surface as dry powders at rates to give 140 ppm on a weight basis of oven-dry soil. Potassium chloride was added at a rate to give 105 ppm of additional K, and NH_4NO_3 and 10-10-10 at rates to give 50 ppm of additional N to the soil.
[b] Based on scale in which 0 indicates all roots apparently healthy and 100 all roots and epicotyl rotted.
[c] Symbols denote relative appearance of aboveground pea plants: 0 = all plants dead; ++++ = all plants healthy.

Table 3. Root rot severity, relative plant height, and appearance of peas as affected by methionine and norleucine (with or without fertilizer) applied during planting to soil subsequently infested with zoospores of *Aphanomyces euteiches*.

Placement and kind of treatment[a]	Disease severity index[b]	Plant height (% of non-infested in each treatment)	Plant appearance[c]
Two inches below the seed:			
Methionine	73	81	++
Methionine + 10-10-10	85	56	+
Norleucine	87	69	++
Norleucine + 10-10-10	88	72	++
10-10-10	93	64	+
None (infested control)	96	51	+
At the same level as the seed:			
Methionine	69	87	++
Methionine + 10-10-10	45	80	+++
Norleucine	73	74	+
Norleucine + 10-10-10	40	96	++
10-10-10	83	90	++
None (infested control)	95	70	+

[a] The amino acids were added as dry powders at rates to give 140 ppm on a weight basis of oven-dry soil, and 10-10-10 was used at a rate to give 50 ppm of additional N to the soil.
[b] Based on scale in which 0 indicates all roots apparently healthy and 100 all roots and epicotyl rotted.
[c] Symbols denote relative appearance of aboveground pea plants: 0 = all plants dead; ++++ = all plants healthy.

ids were ineffective (Fig. 1).

Penetration of pea roots by A. euteiches takes place in less than 24 hours from the time zoospores are placed in the immediate vicinity of roots (1). The relatively short time required for penetration, and the fact that very little or no control is achieved when drenches or side-dressings are delayed 3 or more days after soil infestation, indicates that the amino compounds interfere with the early stages of disease development. Since no detrimental action of some of the amino compounds on zoospores is known (7, 8), it seems possible that these chemicals reduce the severity of the disease by preventing penetration into the root or the early spread of the fungus in the tissue. The fact that there was some control of the disease even with applications during planting (10 to 12 days before soil infestation) indicates residual action in the soil or through the plant. Methionine and a few other amino compounds recently were found to be readily absorbed by pea roots and translocated upwards (3).

Several workers (2, 4, 5, 10) reported that N fertilizers reduced damage caused by A. euteiches on peas. Wade (11) obtained considerable disease reduction by applications of K to a soil with a low salt content. In the present studies, the fertilizers were less effective than the two amino acids in reducing root rot. Combinations of amino compounds with fertilizers, however, increased the effectiveness of the amino acids. The synergistic action of amino compounds and fertilizers is of interest since synthetic fertilizers are used extensively for growing peas commercially.

The inoculum concentrations used in most of the present experiments (about 1.5 million zoospores/can) were probably higher than those commonly encountered in infested fields. Inoculum concentration in the "naturally" infested soil used was presumably lower than that in the artificially infested soil. This may account for the high effectiveness of methionine and norleucine in reducing root rot in the naturally infested soil at relatively low concentrations, even when the two amino acids were applied during planting.

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CHEMICAL CONTROL OF RHIZOCTONIA SOLANI AND SCLEROTIUM ROLFSII OF SNAP BEANS IN BOCA RATON, FLORIDA, 1962[1]

Huey I. Borders[2]

Summary

DAC 649 and Hercules 3944 gave statistically significant control of Rhizoctonia root rot of snap beans caused by Rhizoctonia solani and of southern blight caused by Sclerotium rolfsii. Hercules 3944 gave statistically significant control of Rhizoctonia solani at the 1% level and of Sclerotium rolfsii at the 5% level of significance. DAC 1200 was relatively ineffective.

INTRODUCTION

Experiments for the control of Rhizoctonia root rot and southern blight of snap beans have shown that certain materials will give varying degrees of control of these diseases, but none has been as successful as desired by the growers of snap beans on the sandy soils of the Florida Lower East Coast. The test reported here was conducted in an effort to find a more effective and practical treatment to control these soil-borne fungi.

MATERIALS AND METHODS

Treatments were replicated four times in 1/200-acre plots in randomized complete blocks. All plots received 500 pounds of 6-8-8 fertilizer per acre. Treatments were applied on February 27, 1962 as in-furrow drenches using 400 gallons of water per acre for each drench. The drenches were applied immediately before planting Harvester variety snap beans. Materials used and rates per acre are shown in Table 1.

Table 1. Soil treatments applied for control of soil-borne pathogens of bean plants, 1962.

No.	Material and rate active per acre (in pounds)	Number of plants (means)	No. of Sclerotium rolfsii infected plants (means)	No. of Rhizoctonia infected plants (means)
1	DAC 649-50WP[a] -4	244.50	0	0
2	DAC 649-50WP -6	231.75	0	.50
3	DAC 649-50WP -8	179.00	0	0
4	DAC 649-50WP -2	237.50	1.25	1.50
5	Hercules 3944-50WP -2	229.75	1.00	.25
6	DAC 1200-50WP -2	223.25	5.25	2.25
7	DAC 1200-50WP -4	244.25	2.00	.75
8	Check - no treatment	222.75	3.75	2.50
	LSD .05	44.41	2.08	1.47
	LSD .01	60.45	2.83	2.00

[a] DAC 649 is a product of Diamond Alkali Company, Cleveland, Ohio. The chemical is 3,3,4,4,-tetrachlorotetrahydrothiophene-1-1-dioxide.
DAC 1200 is a product of Diamond Alkali Company, Cleveland, Ohio. The chemical is 2-chloro-p-xylene dithiocyanate.
Hercules 3944 is a product of Naval Stores Department, Hercules Powder Co., Wilmington, Delaware. The chemical is 5-chloro-4-phenyl-1,2-dithiol-3-one.

Emergence and stand of bean seedlings were normal in all treatments except No. 3; DAC 649 used at 8 pounds active per acre caused some reduction in stand (Table 1). On March 20, diseased and total number of plants in the plots were counted.

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This test shows that the incidence of Rhizoctonia infections was reduced with DAC 649 at rates of 4, 6, or 8 pounds active per acre and with Hercules 3944 at 2 pounds active per acre.

Sclerotium rolfsii infections were controlled with DAC 649 at dosages of 4, 6, or 8 pounds active per acre. Less control was obtained with Hercules 3944 at 2 pounds active per acre, and with DAC 649 at 2 pounds active per acre.

Further tests will be conducted to find the optimum dosage of DAC 649, and to find a more effective dosage of Hercules 3944.

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CHEMICAL CONTROL OF BACTERIAL AND CERCOSPORA LEAF SPOTS AND PHYTOPHTHORA BLIGHT OF PEPPER ON SOUTH FLORIDA SANDY SOILS[1]

Huey I. Borders[2]

Summary

A mixture of captan, tribasic copper sulfate, and pentachloronitrobenzene applied as an in-furrow soil drench at planting and as a plant and soil spray at one week intervals during the growing season gave outstanding control of root-rot and aerial infections by Phytophthora capsici and of leaf spots caused by Xanthomonas vesicatoria and Cercospora capsici.

INTRODUCTION

For the past 8 to 10 years Phytophthora capsici Leonian root rots of pepper have caused extensive losses to the vegetable growers on the sandy soils of the Lower Florida East Coast. In 1958-59 approximately 90% of 1900 acres of peppers in Broward County were destroyed by root rots (3).

Previous work on Phytophthora capsici infections of peppers has been directed toward control of the soil-borne plant pathogens. Except during periods of rainy weather or under overhead irrigation, these infections seemed to arise mainly from invasions of the root systems by soil-borne mycelia and oospores. However, even during dry, sunny weather dew formations and the high water table resulting from row irrigation leave the foliosphere and the soil beneath the plants moist and cool for many hours. This condition is favorable for the production of sporangia and the consequent invasion of the aerial parts of the plants. In many pepper fields Phytophthora infections originating in the axils and on the stems were observed to progress rapidly down the main stems of the plants. Within a few days the appearance of these plants was indistinguishable from that of plants in the same fields that had died from soil-borne infections. Foliar sprays were, therefore, added to soil treatments in this experiment.

MATERIALS AND METHODS

The experiment was laid out in four randomized complete blocks on land where previously planted pepper crops had been heavily infected with Phytophthora capsici, the bacterial leaf spot organism Xanthomonas vesicatoria (Doidge) Dows., and the fungus leaf spot caused by Cercospora capsici Heald & Wolf. Each plot consisted of 1/200 acre and contained two plant rows 20 inches apart on beds 66 inches wide, in line with commercial practice.

Two materials were tested in this experiment. One of them, Monsanto 30249[3], is recommended by the manufacturer as a soil treatment, so was used in-furrow only. The other,

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[3] Monsanto 30249 = 2-chloro-3-(tolylsulfonyl) propionitrile furnished by Monsanto Chemical Co., St. Louis, Missouri for use as a soil treatment.

EF 1261-960[4], has been tested at Plantation Field Laboratory since 1960 as both a soil treatment and a spray. In 1961 (1) it gave statistically significant control of soil-borne infections of Manalucie tomatoes caused by the new pathogenic race of Fusarium reported by Stall (4). The components of this mixture have been used as fungicides and bactericides, and a mixture of captan and pentachloronitrobenzene (2) has been widely used as a control for pepper root rots caused by Rhizoctonia solani Kuehn and Pythium spp. However, neither the individual components nor the mixture of captan and pentachloronitrobenzene has shown the effectiveness of EF 1261-960 in controlling Phytophthora root rots and leaf spots of pepper. EF 1261-960 is a mixture of 50% captan, 53% tribasic copper sulfate, and 75% pentachloronitrobenzene used in 3:4:2 proportions. This mixture was applied as an in-furrow soil treatment at planting time, and also, in Treatment No. 1 of Table 1, as a weekly spray-drench to the pepper plants and to the soil beneath the plants. Both the in-furrow soil treatment and the spray-drench were applied at 36 pounds of the mixture in 400 gallons of water per acre. All other treatments shown in Table 1 were applied in-furrow only.

Table 1. Effect of various materials for control of pepper soil-borne and foliage diseases.

No.	Material and rate per acre	No. of plants per treatment (means)	% of Phytophthora-infected plants (means)	Leaf spot disease rating[a]
1.	EF 1261-960 at 36 lb in 400 gal. water. In-furrow and as weekly drench-spray	42.3	0	(trace only)
2.	Monsanto 30249 Liquid at 10 lb in 400 gal. water. In-furrow	40.3	70.9	5
3.	Monsanto 30249 Granules at 10 lb. In-furrow	39.8	67.4	5
4.	EF 1261-960 at 36 lb in 6-8-6 fert. at 1500 lb. In-furrow	38.0	62.2	5
5.	EF 1261-960 at 36 lb. Applied dry. In-furrow	36.8	60.4	5
6.	Monsanto 30249 Granules at 50 lb. In-furrow	35.5	61.9	5
7.	Monsanto 30249 Liquid at 50 lb in 400 gal. water. In-furrow	26.5	68.8	5
8.	Check, no treatment	35.5	78.3	5
	LSD .05	7.5	30.9	
	LSD .01	10.0	42.1	

[a] Leaf spot disease rating, 0-5, based on 0 = no leaves infected, 5 = all leaves with one or more leaf spots.

Soil treatments were applied on November 3, 1961. Florida Giant green pepper seeds were planted immediately following soil treatment. An 8-8-8 fertilizer at 750 pounds per acre was top dressed on the row after planting.

When the pepper seedlings were 6 inches high they were thinned to 9 inches apart in the row. Insecticidal sprays were applied at approximately 1-week intervals[5]. The spray-drenches in Treatment No. 1 were continued at 7-day intervals beginning when the plants were about 3 inches high. Final counts were made on May 9, with postseason observations on May 28.

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[4] EF 1261-960 = a captan-copper-pentachloronitrobenzene material furnished by Everglades Fertilizer Co., Fort Lauderdale, Florida.

[5] The author is indebted to Mr. Thomas I. Whitworth of Pompano Beach, Florida for furnishing the land and for carrying out cultural and insect control practices for this experiment.

RESULTS AND DISCUSSION

The results (Table 1) indicate that EF 1261-960 was effective as a fungicide against *Phytophthora capsici* and *Cercospora capsici* and as a bactericide against *Xanthomonas vesicatoria.* None of the plants treated with EF 1261-960, Treatment No. 1, as an in-furrow treatment followed by sprays showed any Phytophthora symptoms, while from 60 to 78% of the plants in the other treatments were infected.

All the plants in the first three replications of the check were dead as a result of the Phytophthora disease. The few live plants in the fourth replicate were heavily infected by *Phytophthora* and with Xanthomonas and Cercospora leaf spots. The two kinds of leaf spots were present in about equal numbers. Most of the plants in the other treatments were also killed by Phytophthora infections, and the plants that were not killed were heavily infected by *Phytophthora* and the leaf spots. There were only a few small leaf spots in Treatment No. 1, and these were found at the end of the season. There was equally good control of bacterial spot on the fruit. At the end of the season the peppers were allowed to ripen on the plants. The spray applications prevented bacterial and Cercospora spots from developing even on the senescent plants and the fully ripened pepper pods.

Most of the commercial pepper fields in the South Florida area are terminated early in May. At the postseason inspection of the plots on May 28, the plants receiving EF 1261-960 as soil treatment and sprays were still free from Phytophthora symptoms, and only a few leaf spots were found on some of the new foliage. Practically all plants not treated with EF 1261-960 as described were dead. The few that were not dead were very heavily spotted with *Xanthomonas* and *Cercospora*.

CONCLUSIONS

Soil-borne and aerial infections of peppers by *Phytophthora capsici* were controlled by the combination of a soil treatment and spray drenches of EF 1261-960. The same material and method of application also gave a high degree of control of the leaf spots of pepper caused by *Xanthomonas vesicatoria* and *Cercospora capsici*.

The soil-treatment-foliar application approach to the control of soil-borne and aerial diseases caused by these pepper plant pathogens should enable growers to continue to grow profitable peppers on some of the older, disease-infested fields on the Florida Lower East Coast sandy soils.

Subsequent experiments are planned to find the minimum effective dosage and number of spray drenches of EF 1261-960. Other materials will also be tested using this method of application.

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RAMULARIA SCHULZERI ON BIRDSFOOT TREFOIL IN NEW YORK

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Abstract

Ramularia schulzeri Baeumler, previously reported on Lotus purshianus in Nebraska and Texas, was found on seedlings of Lotus corniculatus var. European in New York in 1958. Presence of the fungus was noted by mildew-like spots on some leaves. The fungus was readily cultured on 2% PDA, but attempts to infect L. corniculatus and L. purshianus with conidia from pure cultures were unsuccessful. It is concluded that the fungus is not an especially virulent pathogen.

INTRODUCTION

The only prominent, and occasionally serious, foliage disease of birdsfoot trefoil in New York is that caused by Stemphylium loti Graham (1). Reddish-brown, slightly sunken leaf and stem lesions cause premature defoliation and stem girdling. Stemphylium leaf spots are generally observed on leaves on the lower half of the plants.

White spots, distinctly different from the reddish-brown lesions caused by S. loti, were found on leaves on the upper half of a few 6-month-old L. corniculatus var. European plants in Tompkins County, New York on September 5, 1958. The conidiophores of the causal fungus emerged through the stomata in a fascicle which produced small, lens-shaped, white, mildew-like spots. The leaflets were otherwise green and healthy in appearance, with no visible necrosis or yellowing adjacent to the stomata from which the conidiophores emerged.

RESULTS

Conidia from the white spots were cylindrical, hyaline, single or catenulate, usually nonseptate but occasionally 1-septate, and measured 14 x 3.5μ. The fungus was isolated on 2% PDA and on oatmeal agar. Conidial measurements of the fungus growing on 2% PDA were 13.5 x 3.5μ. Conidial measurements from both sources and the fasciculate conidiophores were within the limits described by Saccardo (2) for R. schulzeri and were essentially identical to the herbarium specimen studied[2]. Measurements of KOH-soaked conidia from the fungus in leaves of the herbarium specimen were 13.7 x 3.8μ.

This is the first report of R. schulzeri occurring on L. corniculatus and in New York State. It has been reported previously on L. purshianus from Nebraska and Texas (3).

Since R. schulzeri apparently occurs only rarely, an evaluation of its pathogenicity was made. Foliage of 1- to 2-month-old birdsfoot trefoil seedlings, varieties European and Viking and L. purshianus, was inoculated by atomizing a conidial suspension of R. schulzeri which contained a drop of Tween 20 as a wetting agent. In three separate experiments, the plants were incubated at constant temperatures of 65°, 72°, and 80° F, respectively, at near 100% RH for 72 hours with a day-length of 16 hours. Even though no visible symptoms appeared within 2 weeks, reisolation attempts were made on 2% PDA. The fungus was never recovered from inoculated plants. Presumably, optimum conditions for infection were not maintained during these tests.

Samples were taken every 12 hours up to 72 hours from the experiment conducted at 72° F and the leaves were cleared in Carnoy's solution to facilitate observation of conidial germination and possible germ tube penetration. Conidia germinated within 12 hours. Hyphae ramified on the surface of the foliage and formed club-shaped appressoria, but subsequent penetration and infection were not observed in either of the Lotus species.

The fungus was not found the following year in the planting from which it was originally collected, nor has it been observed through 1961 on any new or established plantings of birdsfoot trefoil in New York[3].

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[2] Specimen Bartholomew Fungi Columbiani 2680 in the Cornell University Plant Pathology Herbarium, Ithaca, New York.

[3] 1961 observations by Dr. Roy L. Millar, Assistant Professor of Plant Pathology, Cornell University, Ithaca, New York.

DISCUSSION

The late season occurrence of *R. schulzeri* on birdsfoot trefoil and the failure to prove its pathogenicity on vigorous plants suggest that the fungus probably is not an especially virulent pathogen. *R. schulzeri* appears to pose no threat to birdsfoot trefoil in New York during normal growing seasons.

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YIELD REDUCTIONS AND STALK ROT SEVERITY IN CORN HYBRIDS UNDER SEVERE HELMINTHOSPORIUM LEAF BLIGHT CONDITIONS IN PENNSYLVANIA[1]

C. C. Wernham[2], Walter I. Thomas[3], and George W. Gorsline[4]

Summary

Single season trials indicate quite variable losses due to Helminthosporium spp. leaf blight measured by the differences in yield between duplicate plots, one inoculated six times, the other field run.

Early hybrids displayed losses from 35.9 to 76.7%; midseason hybrids exhibited a range from 18.0 to 53.3%; in late hybrids the range was 3.9 to 55.4%. The corresponding ranges for erect (stalk rotted) plants were: early 6.7 to 57.3%; midseason 13.3 to 90.7%; and late 0.0%. Good leaf blight resistance is not always correlated with good stalk rot resistance. Evidence is offered to show that as the corn plant increases in maturity, its susceptibility to leaf blights increases.

The purpose of this study was to evaluate the resistance to common leaf blights of corn hybrids offered for sale to farmers in Pennsylvania. There are two common leaf blights of corn in Pennsylvania, northern leaf blight caused by Helminthosporium turcicum (Trichometasphaeria turcica Luttrell), and southern leaf blight caused by Helminthosporium maydis (Cochliobolus heterostrophus Drechs.). Dried ground leaf material used in inoculation experiments in the disease nursery and in the field carries inoculum of both pathogens. Weather conditions largely determine which leaf disease becomes prominent during the season. H. turcicum is enphytotic throughout the corn growing areas of the State; enough infection is produced each year to furnish inoculum for the succeeding one. H. maydis is common in the southern counties but the northern limit of its occurrence is dependent upon the season. Hot dry weather during any part of the growing season favors its appearance.

Yield losses due to each of these leaf blights have been reported by Ullstrup (8, 9). Yield losses due to both organisms, in a year more favorable to northern leaf blight, on a wide range of commercial, Experiment Station and experimental hybrids are reported here.

MATERIALS AND METHODS

The hybrids were commercially available ones which are widely used in Pennsylvania, except for some entries which were experimental double crosses made with one or more blight-resistant lines.

In general, the results are from duplicate plantings of an unbalanced lattice design of three replications, one inoculated, one non-inoculated and grown sufficiently distant in the experimental area to avoid inoculum drift. In the case of the early hybrids (Table 1) only the inoculated plot was grown on the experimental area. The non-inoculated "data" represent the mean performance of the hybrids grown at two population densities at three locations in the northern part of the State -- their adapted area.

The term non-inoculated means leaf blight under natural occurrence. The inoculated plots received a pinch of ground leaf inoculum once a week for 6 successive weeks beginning June 6 at Landisville, Pa. (planted May 3) and June 20 at Centre Hall, Pa. (planted May 19). Disease ratings were taken three times on the late hybrids but only once on the early and midseason groups. The rating scale used was that recommended by Ullstrup, et al. (10), that is, 0.5, 1.0 ---- 5.0, in which 5.0 represents complete susceptibility. The percentage of erect plants was ascertained by counting stalks broken below the ear. The vast majority of these plants were broken as a consequence of stalk rot, which was no doubt intensified by the destruction of leaf surface as pointed out by Holbert, Hoppe and Smith (3). There was relatively little damage by corn borers in the 1961 season. Harvesting of the non-inoculated plots was by picker-sheller, but the inoculated plots were harvested by hand, since there were no erect stalks in the inoculated late hybrid group. Variations in maturity were measured by percentage moisture at

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[2] Professor of Plant Pathology.

[3], [4] Associate and Assistant Professor, respectively, of Agronomy.

Table 1.

	Combined leaf blight rating			% erect plants	% yield losses
Early hybrids					
range		3.1 - 4.7		6.7 - 57.3	42.2 - 76.7
mean		3.79		26.5	55.09
correlation		"r"			
	1.2	0.350; Significance "r"			
	1.3	0.827; 5% 0.444			
	2.3 -	0.557; 1% 0.561			
Intermediate hybrids					
range		2.7 - 4.2		13.3 - 90.7	18.0 - 62.4
mean		3.52		49.9	40.9
correlation		"r"			
	1.2	0.671; Significance "r"			
	1.3	0.659; 5% 0.367			
	2.3	0.304; 1% 0.470			
Late hybrids	1a Aug 9	1b Aug 24	1c Sept 13		
range	1.2 - 3.3	2.5 - 3.8	4.0 - 5.0	0.0 - 0.0	3.2 - 55.4[a]
mean	2.42	3.15	4.84	0.0	31.5
correlations	"r"				
	(1a:3) 0.652	(1b:3) 0.815	(1c:3) 0.539		
		Significance "r" 5% 0.288 1% 0.372			

[a] The mean % yield loss decreases with each maturity class.

harvest and silking dates.

RESULTS AND DISCUSSION

General: Had the non-inoculated plots been hand harvested, yield differences between the inoculated and non-inoculated might have been greater. The picker-sheller was used to reduce harvesting costs and for agronomic reasons to judge hybrid performance on a farm-harvesting basis. However, the reverse procedure would have exaggerated the losses of the inoculated plots due to the high percentage of non-erect plants. Pennsylvania is a corn importing State and it is imperative that the acreage devoted to maize produces at the greatest efficiency.

Early and Midseason Hybrids (Table 1): Only 6 of the 20 early inoculated varieties displayed less than 50% loss in yield, compared with the naturally infected entries, the range being 35.9 to 76.7%. These losses exemplify the remarkable difference in tolerance in corn hybrids under severe blight conditions, an observation that holds true in all the maturities tested. Little attention has been given to leaf blight resistance in early and midseason lines, so that great differences in blight reaction are not to be expected.

Of the above six hybrids, four exhibit the highest percentages (57.3, 50.0, 46.7 and 44.0) of erect or non-stalk rotted plants. These same four hybrids yielded 92.7, 87.3, 89.0, 89.0 bushels/acre. This combination of high yield plus tolerance to leaf blight infection plus good standability (comparatively) should place four of these on the highly recommended list.

The combination of standability and blight resistance with acceptable yield might be most quickly accomplished by using the single major gene sources for blight resistance reported by Van Schaik and Le Roux (11) and Hooker (4) as the non-recurrent parents in (blight resistant x standability) crosses. It is too early to state that these major genes are effective against all strains of *H. turcicum*. Le Roux has indicated[5] that the South African sources carrying major genes of Mexican origin are not universally resistant. The reaction to *H. maydis* is also unknown, but for early hybrids outstanding resistance to southern leaf blight is not required.

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[5] A personal communication to the senior author.

The midseason hybrids, which usually reflect some lines in common with later hybrids, were rather disappointing. Only one entry displayed acceptable leaf blight resistance, high percentage of erect plants and high yield, the figures being respectively 2.7, 82.0 and 117.3. Obviously much remains to be done in this group both from the standpoint of standability and leaf blight resistance. This group too exhibited a wide range of tolerance to severe leaf blight infection.

Late Hybrids (Table 1): The data on the late hybrids is most interesting for the following reasons: 1) The late hybrids are representative of the best efforts of corn belt maturity breeding; 2) more effort has been spent on producing blight resistant inbreds for this maturity class; 3) there were no erect plants in the inoculated plots at harvest time; and 4) for the first time statistical evidence exists to show that as the maize hybrid matures, leaf blight susceptibility increases.

The late hybrids were both exciting and disappointing. Two commercial and one experimental hybrid were high yielding (135.2, 129.6, 116.3 bushels/acre) with low percentage loss due to disease (7.8, 7.1, 3.2) compared with seven other high yielding hybrids (138.8, 140.7, 136.1, 134.5, 136.4, 143.7, 151.4 bushels/acre) with much higher percentage losses (38.5, 40.1, 22.9, 44.5, 50.2, 43.5, 29.5). The range of tolerance due to disease was even greater.

The disappointment with the poor standability (high stalk rot susceptibility) cannot be overemphasized. The stalk rot problem is undoubtedly the top priority concern of corn pathologists and breeders (1, 2, 5, 6, 7). Techniques for testing and selecting inbreds are numerous but all appear to have serious shortcomings.

These studies suggest that if selection of inbred lines and of hybrids between them were made under conditions of great stress (high population densities, high incidence of leaf blight coupled with good fertility and ample moisture) in the region of their adaptability, the selection of erect (resistant) plants could be increased without undue expense in labor and experimentation.

Evidently, leaf blight resistance does not automatically confer lodging (stalk rot) resistance, a fact frequently noticed in the maize breeding nursery at University Park, Pa. Also, stalk rot readings on late segregates in this nursery are poor indicators of stalk rot performance in a long season environment. Ideal selection pressures occur only in a full season for maturity, probably because stalk rot is an old age disease of corn. In these experiments loss of viable leaf surface without doubt contributed to the senescence of the hybrid corn plants. Preharvest data indicated that the four hybrids most resistant to leaf blight remained erect the longest.

For years we have been concerned with the effect of age of plant on leaf blight ratings. Two types of evidence were continually confronting us: (a) Our blight readings were often more severe than those taken by other observers in Florida where leaf blight is almost annually epiphytotic, and (b) under field conditions one frequently met with cases wherein a canopy of leaf blight existed on the leaves above the ear, the lower ones being quite free from infection. Were these plants resistant or susceptible? Our southern colleagues, rating corn in Florida, were of the opinion that leaf blight reached its maximum expression shortly after silking and maintained that level until the grain was fully matured. The ever present threat of early frost prevented an exacting investigation of this phenomenon at University Park. Such danger does not exist at Landisville, Pennsylvania.

A "t" test analysis[6] showed conclusively that there was more blight per hybrid with each successive reading, but the reading at any point of time was significantly correlated with yield loss. The data indicate that under a uniformly progressive leaf blight epiphytotic, corn in a nursery of mixed maturities should be rated in order of maturity from early to late. Under local conditions in which late July and early August weather is likely to be quite dry and one has to await the heavy dews characteristic of the late summer, blight buildup is not likely to be severe until September. At such time early selections are rapidly approaching senescence and therefore more susceptible. The breeder would therefore have to adopt a different standard of performance for the early maturity class.

CONCLUSIONS

It would be foolish to leave the impression that the losses demonstrated in these 1-year, one-season trials are representative of annual blight losses of corn in Pennsylvania. We have not had a statewide epiphytotic of leaf blight since 1942. However, very few seasons go by in which some farmer or group of farmers in some low-lying valley or creek bottom has not suffered some loss due to leaf blight. To him as an individual the loss is economically as real as though blight were statewide. Admittedly his loss averaged with those of his non-stricken confrères is negligible as a statewide statistic and too frequently leaf blight resistance is looked upon as an unworthy objective in corn breeding work.

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[6] By L. D. Moore, Research Assistant.

The results recorded here merely indicate what might happen if blight resistance is completely ignored. The epiphytotic of 1942 had positive selective value in that some very susceptible hybrids like Ia 939 and Ia 4059 were dropped from the recommended list, not only because of their blight susceptibility but of the attendant phenomenon of badly lodged plants. Stalk rot is annually our most persistent and troublesome disease so that it becomes good logic to hold to a minimum any factor such as leaf blight that may complicate the problem.

There is the additional fact of blight tolerance as measured by the effect on yield. Certainly leaf blight and stalk rot resistance is not going to be resolved immediately, but tests conducted on the basis of those listed above can do much to remove from the commercial scene those hybrids that are least tolerant to the total disease syndrome.

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RHIZOCTONIA BLIGHT OF TALL FESCUE GRASS[1]

E. S. Luttrell

Abstract

Although isolates of Rhizoctonia from various forage grasses and legumes varied widely in growth rate and colony morphology, intergradations made classification into cultural types difficult and of questionable value. The isolates differed in level of pathogenicity on all hosts, but showed no host specificity. All isolates were placed in Rhizoctonia solani Kuehn (Pellicularia filamentosa (Pat.) Rogers). The most effective method of inoculation was dusting moistened plants with ground dried oat grain cultures. Inoculation of leaves with whole grains from oat grain cultures yielded quantitative data in terms of lesion diameters from single-point inoculations. Clonal lines from tall fescue plants that appeared highly resistant in the field proved to be susceptible under artificial inoculation.

INTRODUCTION

Blight caused by Rhizoctonia solani Kuehn is the most serious disease of tall fescue grass (Festuca elatior var. arundinacea), the best adapted perennial pasture grass for year-round grazing in many parts of the Southeast. Since tall fescue is deficient in forage quality, it must be improved by breeding if its potential value is to be realized. In conjunction with a breeding program at the Georgia Experiment Station, a study was made of variation in R. solani and of sources of resistance to blight in tall fescue.

Characteristic symptoms on tall fescue leaves are large, irregular, tan to whitish blotches with narrow, dark-brown borders. Under warm, moist conditions, the fungus spreads rapidly through contact from leaf to leaf. Although the crowns of established plants often survive, the foliage may be reduced to a sodden mass covered with a web of mycelium. Ladino clover, which is commonly interplanted with tall fescue, is equally susceptible to aerial infection with R. solani. The perfect stage of the fungus, Pellicularia filamentosa (Pat.) Rogers, is not uncommon on tall fescue and Ladino clover in the form of effuse white patches, usually on the lower surface of the leaves. The leaf tissue remains green and apparently is unaffected by the growth of the fungus in the perfect stage.

ISOLATIONS AND CULTURAL TYPES

Isolations from basidiospores were made by fixing leaves on which the fungus was fruiting to the lids of Petri dishes so that the basidiospores were discharged onto the agar below. Since R. solani is frequently killed by the usual procedures of surface sterilization, isolations from infected leaves, stems, and roots were made by washing bits of the tissue in sterile water and planting the fragments on water agar plates. Isolates grown on potato-dextrose agar plates at 28°C were classified into the following types:

Type A - Surface mycelium thick, compacted, stromatoid, white to tan, crossed by strands of dark-brown hyphae; aerial mycelium sparse, white to tan; sclerotia small (1 mm diam.), flat, tan to brown, formed on the surface of the colony in lines radiating from the point of inoculation, at the margins, and in the aerial mycelium; growth rate 0.5-0.7 mm/hour; 7 isolates from tall fescue, Ladino clover, Illahee red fescue, smooth brome grass, and grain sorghum (stalk rot).

Type B - Surface mycelium sparse, tan; aerial mycelium sparse, white to tan; sclerotia large (1-4 mm diam.), smooth, globular, dark brown, scattered over the surface of the colony; growth rate 0.7-0.9 mm/hour; 7 isolates from tall fescue, Ladino clover, and pearl millet.

Type C - Surface mycelium thick, white; aerial mycelium dense, white; sclerotia essentially lacking, represented by tiny hyphal wefts in the aerial mycelium; growth rate 0.1-0.5 mm/hour; 11 isolates from tall fescue, Ladino clover, crimson clover, trefoil, rye grass, and orchard grass (root rot); basidiospore isolates from tall fescue and trefoil.

Type D - Surface mycelium sparse to moderately thick, white to brown; aerial mycelium sparse to dense, white to tan; sclerotia small to large, irregular, gray to brown, often confluent, formed usually at the margins of the colony; growth rate 0.5-1.1 mm/hour; a large and variable central group remaining after segregation of the more distinctive isolates and intergrading with all other types; 57 isolates from tall fescue, Ladino clover, trefoil, rye grass, smooth brome grass, Johnson grass, redtop grass, Bermuda grass, carpet grass, and rescue grass; basidiospore isolates from tall fescue and Ladino clover.

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[1] Journal Series Paper No. 427, Georgia Experiment Station.

In Table 2 growth rates are recorded in terms of colony diameters after 48 hours to afford a comparison with size of lesions produced on host leaves in the same length of time.

METHODS OF INOCULATION

Inoculations were made with a single Type B isolate on fully expanded leaves of tall fescue and soybean and on 2- to 3-week-old seedlings of tall fescue, rye grass, Ladino clover, and trefoil grown in pots of autoclaved soil. Inoculated plants were kept in an inoculation chamber at approximately 28°C for 48 hours and returned to greenhouse benches where results were recorded after 2 to 3 days (Table 1).

Spraying plants with a suspension of macerated agar plate cultures (Method 1) gave fairly satisfactory results on tender seedlings and on soybean leaves but was ineffective on mature leaves of tall fescue plants, on which the lesions were limited to flecks and tiny blotches. Addition of a nutrient base in the form of alfalfa leaf meal (Method 2) increased infection. However, the most effective and convenient method was dusting moistened plants with ground dried oat grain cultures (9). Inoculations with single oat grains from whole oat grain cultures (Method 4) or with single sclerotia (Method 5), although tedious, permitted quantitative comparisons in terms of size of lesion produced from a single point of inoculation during a specified period in the inoculation chamber.

PATHOGENICITY

Series of inoculations were made with 31 isolates on the hosts listed in Table 2. Snap bean seed were planted at the rate of 10 seeds per pot in autoclaved sandy loam soil inoculated with 2 tablespoonsful of oat grain cultures. Results in percentage of plants failing to emerge or dying were taken after 6 weeks on greenhouse benches. Mature leaves were inoculated by placing a single contaminated oat grain in the center of each of 10 leaves. Results were recorded as longest diameter of each lesion. Seedling plants were thinned to 10 seedlings per pot and inoculated similarly by placing a contaminated oat grain at the base of each plant. Results were recorded as percentage of plants killed. With the exception of some of the inoculations on leaves of smooth brome grass and soybean, all tests were replicated three times.

Results with a representative series of isolates are in Table 2. Isolates varied greatly in level of pathogenicity on all hosts but showed little indication of host specificity. All Type A and Type B isolates were highly pathogenic, Type C isolates were weakly pathogenic, and Type D isolates varied from highly to weakly pathogenic.

Table 1. Comparison of methods of inoculation with Rhizoctonia solani on mature leaves and on seedlings of grasses and legumes.

	% infection					
	Mature leaves		Seedlings			
Method of inoculation	tall fescue	soy-bean	tall fescue	rye grass	Ladino clover	trefoil
1[a]	22	80	26	27	25	88
2	74	95	25	30	25	85
3	88	100	100	100	100	100
4	93	--	--	--	--	--
5	100	--	--	--	--	--

[a] 1 - Sprayed with suspension of one potato-dextrose agar plate culture macerated in 100 ml of water.

2 - Same spray followed by dusting with alfalfa leaf meal.

3 - Ground dried whole oat culture dusted on plants previously sprayed with water.

4 - Single oat grains from whole oat grain culture dipped in water and placed individually on each leaf.

5 - Whole sclerotia from oat grain culture placed individually on each leaf.

Table 2. Cultural type, growth rate, and pathogenicity of isolates of Rhizoctonia solani.

			Colony	Pathogenicity								
		Cul-tural	diam. at 48 hrs	Leaf blotch-diam. (in mm)			Seedling blight (%)					
Isolate	Host	type	(in mm)	tall fescue	smooth brome	soy bean	rye grass	alfalfa	Ladino clover	trefoil	snap bean
532	Ladino clover	A	61	28	26	24	60	87	100	93	100
577	Grain sorghum	A	64	42	53	46	97	97	100	73	100
509	Tall fescue	B	90	65	55	58	87	93	70	83	100
712	Pearl millet	B	89	57	82	61	50	100	90	77	73
536	Ladino clover	C	43	0	0	0	10	13	0	7	27
543	Rye grass	C	38	0	0	--	0	0	0	10	20
513	Tall fescue	D	91	34	47	44	43	93	100	63	37
518	Trefoil	D	90	16	33	--	37	100	90	70	33
672	Rye grass	D	81	36	42	26	37	73	40	43	70
711	Ladino clover	D	61	20	42	45	30	53	70	7	40
704	Bermuda grass	D	43	6	5	0	20	0	0	0	37

RESISTANCE IN TALL FESCUE

Field selections for resistant plants were made in a spaced planting of clones of tall fescue plants varying in growth habit, leaf width, and coarseness that had been assembled in the breeding program. Under conditions of severe infection, several of these clonal lines appeared to be immune. Clones of three plants each were established in pots from each of five resistant selections and five highly susceptible plants. Each plant was inoculated by placing a contaminated oat grain on each of 10 leaves. On the five clones that appeared resistant in the field mean lesion length on 30 leaves varied from 35 to 46 mm with a grand mean for these clones of 40 mm. On the five clones that appeared highly susceptible in the field mean lesion length varied from 39 to 48 mm with a grand mean for these clones of 44 mm.

DISCUSSION

Although a classification of the isolates into four cultural types was made, it was possible to do so only by recognizing small groups of extreme forms (Types A, B, C) and leaving a large central group of variable forms (Type D) that intergraded with all other types. Classification of borderline forms was difficult. It is more difficult to relate this classification to those proposed by others. Of the types described by Houston (6) it is possible to make a tentative identification only of my Type A with Houston's Type A. No restriction in host range could be related to cultural type since all types were isolated from tall fescue and Ladino clover, the hosts most intensively sampled. Furthermore, there was little correlation between cultural type and pathogenicity except for the fact that forms in Type A and B with well-developed sclerotia were consistently more pathogenic than forms lacking sclerotia in Type C. Variation was in level of pathogenicity on all hosts, not in host range. The general conclusion reached by Kernkamp (7) that R. solani is an extremely variable species composed of an indefinite number of races differing in cultural characteristics and pathogenicity can not be improved upon. Subsequent reports (4, 10) of races with restricted host ranges indicate little possibility of classifying them into formae specialis. In work with a particular crop it is desirable to determine the range of variability existing in the pathogen; it seems impossible to arrange the variants in any practicable scheme of classification.

Since publication of Kernkamp's (7) review, methods of producing the perfect stage of R. solani in culture have been developed (2, 5, 10, 12). This has led to the recognition of two species, Pellicularia filamentosa and P. praticola (Kotila) Flentje (2, 5, 13). However, Flentje's (2) report of variation in sterigma length in P. filamentosa invalidated this criterion, which had been emphasized by Kotila (8) and Hawn and Vanterpool (5) in separating P. praticola. Criteria of growth rates and hyphal diameter suggested by Flentje (2) were invalidated before they were published by Kernkamp's (7) data. Although it has been stated that P. praticola is distinguished by cultural type (2, 11, 13) and suggested that Houston's (6) Type A represented P. praticola (2, 13), variations in descriptions of cultural types of P. praticola (2, 8, 11, 13) make recognition of the species on this basis doubtful. Distinctions are reduced to minute differences in dimensions of basidia and basidiospores and in number of sterigmata most frequently produced on the basidia: 4 in P. filamentosa (range 3-5), 3 in P. praticola (range 1-5). There seems little basis for distinguishing these species at present.

Variability in results with R. solani extends to studies on method of infection, whether by direct penetration (1, 4) or by killing in advance of penetration through toxin formation (7). Regardless of method, infection seems to be influenced by availability of nutrients in sclerotia or in non-living substrates; and this should be taken into account in making inoculations, especially on aerial parts of plants.

No resistance was found in a large collection of tall fescue breeding lines that differed widely in agronomic characters. Results of artificial inoculations indicated that plants showing apparent resistance in the field had merely escaped infection. Attempts to improve forage quality through hybridization with rye grass offer no hope of increased blight resistance since rye grass is more susceptible than tall fescue. Prospects of obtaining blight resistance in tall fescue seem even less encouraging than prospects of improving its forage quality.

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VERTICILLIUM WILT OF SAFFLOWER IN THE UNITED STATES -- A POTENTIAL PROBLEM[1]

D. E. Zimmer[2]

Safflower acreage increased from an estimated 27,000 acres in 1951 to an estimated 500,000 in 1961 (1, 2). This increase resulted in planting safflower in rotation, often with beans, peas, potatoes, tomatoes, and cotton. Since some varieties of these crops are susceptible to Verticillium wilt, incited by Verticillium albo-atrum Reinke & Berth., growing susceptible varieties of safflower in the rotation increases the likelihood of wilt in these crops as well as in safflower.

Schuster and Nuland (3) reported strains of the pathogen, differences in varietal response, and seed transmission in Nebraska.

Verticillium wilt of safflower was observed in limited amounts in 1961 plantings in Idaho, Utah, and Arizona. The disease was found in California in 1962[3]. Symptoms were essentially the same as those reported by Schuster and Nuland (3), except in addition to yellowing on one side of the midrib, diseased leaves usually showed unilateral growth (Fig. 1).

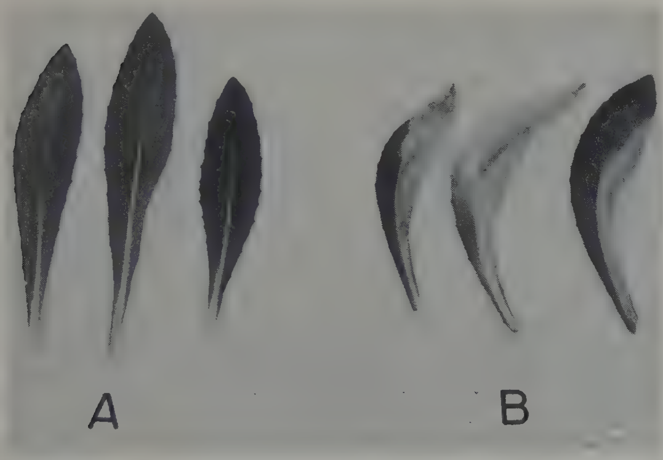

FIGURE 1. A -- Normal safflower leaves. B -- Leaves from Verticillium-infected plants. Note the unilateral discoloration and growth of diseased leaves.

Most present commercial safflower varieties and promising breeding material have not been tested for reaction to Verticillium. Studies were made to determine the susceptibility of 10 varieties and lines, to confirm seed transmission, and to investigate variation in virulence of safflower-attacking isolates.

Isolates from wilted U-1421 safflower grown at Burley, Idaho and N-10 safflower grown at Logan, Utah were obtained. Colony characteristics of these isolates, one isolate from potato, and one from safflower grown in California were determined on four media. Each isolate was used to inoculate 12 seedlings of 10 safflower varieties and lines. Inoculations were performed by root-dipping at the time of transplanting 2-week-old seedlings from flats to greenhouse pots. The disease index of each entry-isolate combination was derived by determining the average number of diseased leaves from the base of the inoculated plants corrected for wilting of healthy plants grown simultaneously.

Of four isolates studied, two were pseudo-sclerotial types and two were mycelial. No correlation was observed between colony type (mycelial or pseudo-sclerotial) and virulence. Schuster and Nuland (3) reported the pseudo-sclerotial type to be virulent on safflower.

Studies showed that greenhouse symptoms parallel field symptoms. Of the entries tested, N-1-1-5 and U-1421-9-16 possessed the lowest average disease index, while Pacific 7, N-4051, and P.I. 253 914 possessed the highest average disease index (Table 1). These and other en-

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[1] Cooperative investigation, Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Utah Agricultural Experiment Station, Logan, Utah. Project No. 474. Journal Paper No. 268.

[2] Research Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Logan, Utah.

[3] Klisiewicz, J. M. 1962. Personal communication.

Table 1. Disease index[a] of 10 safflower varieties or lines 50 days after inoculation with four isolates of *Verticillium albo-atrum*.

Entry	California culture	Potato culture	N-10 culture	U-1421 culture	Mean
Gila	3.7	0.0	5.8	0.5	2.5
N-1-1-5	1.3	0.0	6.7	0.7	1.9
N-6	4.5	3.3	6.3	0.5	3.7
N-10	3.2	3.5	5.3	0.0	3.0
N-4051	5.2	2.2	7.8	0.8	4.0
Pacific 2	3.0	1.3	10.0	1.0	3.8
Pacific 7	6.3	2.2	9.0	0.0	4.4
P.I. 253 914	8.5	4.0	8.3	1.5	5.6
U-1421-9-16	3.2	1.2	3.5	1.3	2.3
U-1421-17	3.3	0.7	7.8	0.8	3.2
Mean	4.2	1.8	7.0	0.7	

[a]The average highest leaf from the base of the plant with wilt symptoms.

tries responded differently to different isolates confirming Schuster and Nuland's (3) findings that isolates of *V. albo-atrum* pathogenic to safflower differ in virulence or aggressiveness.

As reported by Schuster and Nuland (3), Verticillium wilt of safflower was found to be seed transmitted. *Verticillium* was recovered from 53.5% of the seed harvested from greenhouse inoculated plants. In all cases the recovered cultures were identical to the type originally used in the inoculation. Although it has not been demonstrated that seed harvested from Verticillium-infected plants give rise to infected seedlings, seed transmission could be an important means of disease dissemination.

Verticillium wilt is not an important safflower disease. Its potential severity is emphasized by seed transmission, persistence of the pathogen in the soil after introduction, and a wide host range. If Verticillium wilt of safflower becomes an important disease, control through varietal resistance or cultural practices will be necessary. Variability in varietal response indicates that obtaining resistance may be possible. A screening program for resistance to *Verticillium* would necessitate a study of many isolates of *V. albo-atrum* to assure one with maximum or representative virulence.

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DISTRIBUTION, PREVALENCE AND IMPORTANCE OF SOME CANTALOUP VIRUS DISEASES IN SOUTHWESTERN ARIZONA[1]

Merritt R. Nelson[2], Ross M. Allen[3], and Donald M. Tuttle[4]

Summary

A marked decline in spring cantaloup acreage during the past decade in Arizona and California has been attributed to a disease known as "crown blight," cause unknown. Early declining vines (particularly the crown of the plant) and reduced yields are the main effects associated with this disease. Surveys were conducted from 1958-1961 in Yuma County, Arizona to determine the relationship of fields with poor vines and low yields with possible causal agents.

Cantaloup plants infected early and heavily with mosaic virus diseases were those that consistently yielded the fewest fruit, had the poorest vines and were the first to collapse. In the absence of these diseases (watermelon and cucumber mosaics) vine growth was excellent and yields of marketable cantaloups high.

INTRODUCTION

Virus diseases of cucurbits have received much attention recently in connection with the declining spring cantaloup industry in the southwestern United States (2, 3, 4, 7, 8, 9, 11, 12, 13). The decline in spring cantaloup acreage in Arizona and California during the past 10 years has been attributed generally to a condition called "crown blight." While virus diseases of cantaloups have been a recognized problem for 30 years or more (10), crown blight has been considered a major problem for only 15 years. Much effort has been expended to determine the cause of crown blight, but so far no disease-causing agent, cultural factor or combination of such has been demonstrated to be primarily responsible. Indeed, such a complex and profuse list of symptoms has been described for crown blight (for example, marginal, intermarginal and V-shaped leaf necrosis and chlorosis followed by alternate and/or successive petiole collapse with yellow, brown or grey-green type overall collapse) that it has been difficult to find any one factor or factors that will successfully and consistently reproduce the described symptoms. The one effect of crown blight that stands out, however, is the loss of yield due to collapse of the crown of the plant. Insufficient crown foliage results in exposure of the earliest maturing fruit to the sun which renders them unmarketable; the extent and earliness of foliage loss determines the extent of fruit loss.

The main objective of the work reported here is to relate earliness and extent of mosaic infection in cantaloups with yields and vine condition in the fields. Yield and general condition of vines in surveyed fields then are the criteria being used to determine the disease or condition responsible for declining cantaloup yields in Yuma County, Arizona, and hence the factor presumably most important in the so-called "crown blight disease complex."

Yuma County is currently the principal spring cantaloup production area in the United States. While cantaloup acreage in central Arizona and southern California decreased 95 and 75%, respectively, in the past 10 years, Yuma County acreage remained relatively stable at 14,000 acres. The reason is that crown blight outbreaks (and related yield reduction) in Yuma, though severe at times, have not been so frequent or widespread as those in central Arizona and southern California.

Virus diseases of cantaloups and other cucurbits in spring cantaloup production districts include watermelon mosaic (WMV), cucumber mosaic (CMV), squash mosaic (SMV), tobacco ringspot (TRSV), and curly top virus (CTV) (4, 9, 11, 12, 13). Of these viruses only WMV and CMV occur commonly and extensively in Yuma County (11). Curly top is sparse and SMV is only rarely found. Curly top is damaging to cantaloups, especially when infection occurs during early stages of growth (5), but its scarcity makes it of minor importance in Yuma County.

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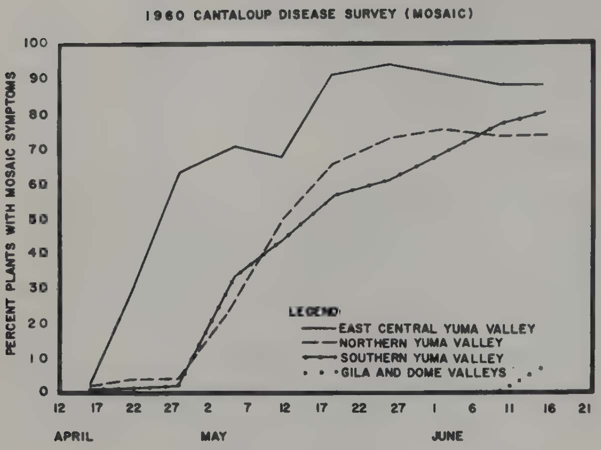

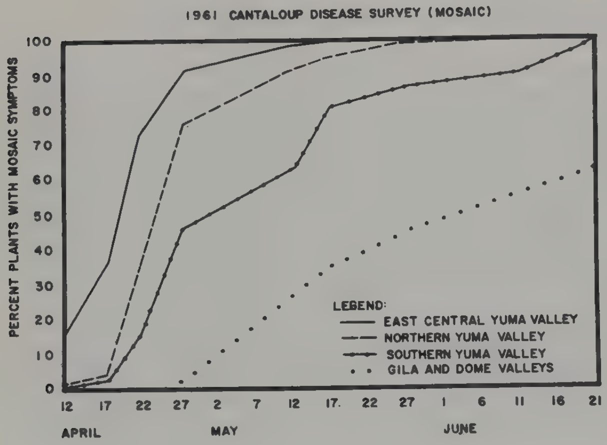

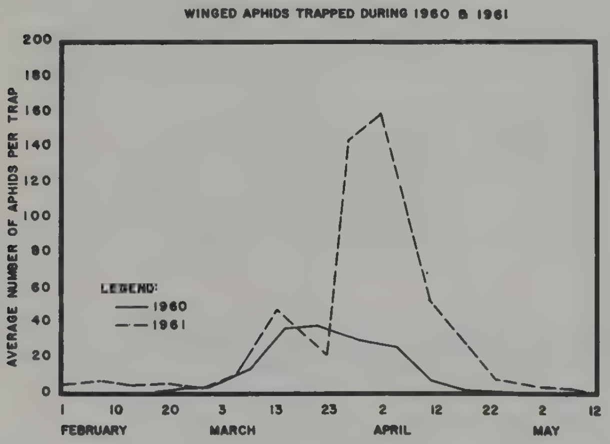

FIGURE 1. Rate of mosaic virus buildup on cantaloups by subareas in Yuma County during 1960.

FIGURE 2. Rate of mosaic virus buildup on cantaloups by subareas in Yuma County during 1961.

FIGURE 3. Rate of winged aphid buildup in Yuma County during 1960 and 1961 (Aphis gossypii, plus Myzus persicae).

Routine surveys during 1958 and 1959 indicated that the part played by mosaic virus diseases in crown blight was important. Therefore, during 1960 and 1961 more extensive surveys were conducted in Yuma County to determine the viruses present and to follow their buildup during the cantaloup season. These data then were used to correlate with yields and rated vine conditions to determine how virus diseases were involved in the "crown blight complex."

MATERIALS AND METHODS

Two types of surveys were conducted. The first was a visual survey of 24 fields, selected early in March, which were uniformly distributed throughout the cantaloup areas. Weekly visits to each field, beginning in March and continuing throughout the cantaloup season (late June), consisted of general observations of diseases present and several random counts of 20 consecutive plants in a single row. Data (Figs. 1 and 2) were recorded of the number of plants either showing or not showing virus symptoms, and from these data the percentage virus incidence was calculated for that week. No attempt was made to differentiate between viruses on the basis of symptoms alone.

The Yuma cantaloup acreage has been subdivided into four areas (Figs. 1, 2, and 4), which have shown definite and consistent differences in rate of mosaic virus increase, final mosaic incidence, and the viruses present. These differences were very striking both years of the survey, despite the fact that total mosaic incidence was distinctly dissimilar in those two years.

In the second type of survey, plant samples showing symptoms were collected for isolation and identification of viruses present. The surveys were made three times during the season and included eight different fields from which four to ten plant samples were collected at random. Plant samples consisted of several inches of a rapidly growing runner tip. Each sample was placed in a plastic bag and kept cool on ice in a portable ice chest. Sap extract inoculum was prepared by grinding the tissue in a mortar and pestle in a 0.1% phosphate buffer (pH 6.8) solution. Inoculations were made to greenhouse-grown diagnostic hosts with cheesecloth pads after adding a small amount of carborundum to the sap extract.

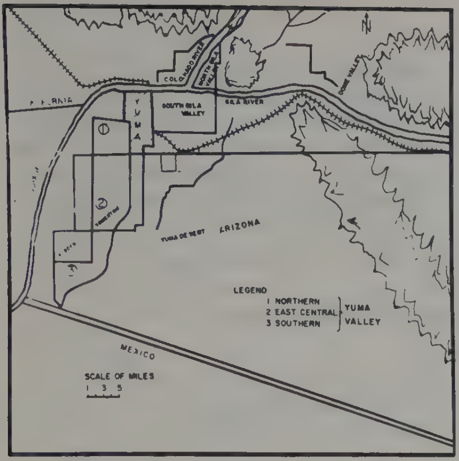

FIGURE 4. Outline map showing relative location of Yuma cantaloup production subareas.

Diagnostic hosts used were *Cucumis melo* (cantaloup cv. PMR-45), *Citrullus vulgaris* (watermelon cv. Klondike), *Nicotiana tabacum* (tobacco cv. Kentucky 57), *Cucurbita pepo* (squash cv. early bush scallop), *Vigna sinensis* (cowpea cv. blackeye), and *Chenopodium amaranticolor* (1961 only).

Viruses present in samples were indicated by test plant reaction as follows: WMV caused a moderate to severe mottle, leaf distortion, stunting and green blisters in cantaloup, watermelon and squash and no reaction on blackeye cowpea, tobacco and *Chenopodium amaranticolor*. CMV produced local lesions on the primary leaves of blackeye cowpea, local lesions on *Chenopodium amaranticolor*, systemic mottle on tobacco, severe chlorotic mottle on cantaloup and squash and chlorotic and necrotic spots on inoculated leaves of watermelon; SMV yielded no reaction on cowpea, tobacco, *Chenopodium amaranticolor* or watermelon, but caused mild veinbanding and mottle on cantaloup with some recovery and severe mottle with green blisters and leaf distortion in squash; TRSV produces necrotic ring patterns in tobacco, local lesions, top necrosis and death of cowpeas and *Chenopodium amaranticolor*, chlorotic stippling and mottle of leaves of cantaloup, squash and watermelon. Tobacco ringspot virus was not isolated from cantaloup in Yuma County.

Mixtures of these viruses were separated in the following manner: WMV was separated from CMV and SMV by transferring the virus from young growing tips of watermelons; SMV was separated from WMV and CMV by dilution series (8); CMV was separated from the other viruses present in Yuma County by passing through tobacco.

Insect prevalence data were obtained weekly by trapping insects on "sticky boards" (6) which measured 10 x 5 inches and were coated on two sides with adhesive. These were placed 4 to 6 feet above ground, two to a location, with a trapping surface oriented in each of the cardinal directions of the compass. Twelve of these trap locations were distributed throughout the agricultural area of southern Yuma County.

RESULTS AND DISCUSSION

The main objective of these surveys was to determine whether mosaic virus disease incidence could be correlated with low yield, early decline and collapse of cantaloup vines. High incidence of mosaic in cantaloups does not necessarily lead to crop failure. Late infections, though still resulting in striking disease symptoms, appear to do little damage. Early infections, however, have been shown to have very severe effects on cantaloup production (12). The data necessary therefore to show the relationship between mosaic virus diseases and "crown blight" or cantaloup decline must indicate both time of infection (in relation to plant growth) and virus disease incidence. These data were obtained for both 1960 and 1961 (Figs. 1 and 2).

Yield records for 2 years show that there is a direct correlation between earliness and extent of mosaic infection and yield of cantaloups. For example, in 1960 (a year of relatively light mosaic infection) three fields in east-central Yuma Valley yielded fewer than 100 crates per acre (average yield 1960 - 170 crates); these same three fields were the first of the 24 surveyed to show mosaic symptoms. In contrast, cantaloup fields in the North Gila Valley and Dome Valley were virtually free from virus infection in 1960 and yielded 261 crates per acre.

Virus disease incidence was higher and occurred earlier in all areas during 1961. Two fields in the east-central Yuma Valley were infected so early and heavily during this season that the melon vines collapsed and died long before harvest. These were the first fields to show mosaic infection during 1961. Infection occurred much later (3 weeks) and built up much slower in the North Gila-Dome area than east-central Yuma Valley in 1961. While appearance was much earlier and buildup of mosaic much greater than 1960 for this area, yields were still near 200 crates per acre because plants were large enough at the time of infection to partially withstand the effects of the virus infection.

Yields in the northern and southern parts of Yuma Valley fall between the extremes represented by North Gila-Dome area and east-central Yuma Valley. Generally, however, the better fields are found in southern Yuma Valley, with yields near or over 200 crates per acre not uncommon.

The results of isolation and identification of viruses from infected cantaloup plants collected at random are shown in Table 1; watermelon mosaic was by far the most prevalent virus during 1960 and 1961 at 95%. Cucumber mosaic, though present in only 22% of the samples, was the most important virus in those fields in which it occurred because it is more severe in its effects on individual cantaloup plants than watermelon mosaic. Plants infected in early stages of growth by watermelon mosaic generally are able to produce some marketable cantaloups. Plants infected by cucumber mosaic in early stages of plant growth, however, are stunted more severely than by corresponding watermelon mosaic infection and produce few marketable fruit (12).

Table 1. Viruses identified in cantaloup specimens from Yuma County, Arizona.

Date	No. test locations[a]	No. isolates obtained	Frequency of recovery of: WMV	CMV	SMV
1960					
April 14	3	11	11	0	0
May 25	8	40	39	6	0
June 15	8	67	66	14	0
1961					
May 26[b]	8	45	40	15	0
June 7	8	24	20	10	0
June 20	8	36	35	4	1
Total	43	223	211	49	1
Percent			94.62	21.97	.45

[a] Test location - single field of 100 or more acres from which random samples of plants showing symptoms were collected.

[b] Although virus symptoms appeared earlier in 1961, isolations were made later than 1960 because of greenhouse renovation and construction during early spring of 1961.

Cucumber mosaic is much more limited in its distribution in Yuma County cantaloup acreage than is watermelon mosaic. The east-central and northern parts of Yuma Valley frequently contain fields heavily infected with CMV while southern Yuma Valley and the North Gila-Dome area rarely or never yield any isolates of this virus. Because CMV is the most destructive virus commonly found in southwestern Arizona and its distribution corresponds with those areas having the poorest yields, the correlation between virus disease and poor yields due to "crown blight" is strengthened.

Mosaic infection in Yuma County appeared earlier and was much more extensive in 1961 than in 1960. Average yield records for these 2 years correlate with this difference in mosaic incidence; average yield was 170 crates per acre in 1960 corresponding to late and low mosaic incidence, whereas, in 1961 an earlier and more rapid increase in mosaic infection resulted in an average yield of only 127 crates per acre.

The abundance of winged aphids in 1961 was strikingly higher than in 1960 (Fig. 3). The first of two peaks for 1961 was higher than the single peak in 1960. Numerically, there were five times as many aphids in 1961 as in 1960 and they appeared earlier and persisted longer. This great abundance of aphids in 1961 corresponds with the early and extensive mosaic infection for that year. Insect data are shown as the sum of the average number of green peach aphids (Myzus persicae) plus the melon aphids (Aphis gossypii) per trap in Figure 3. In 1960 the green peach aphid accounted for 78% of the total while the melon aphid was 22%. Eighty two percent of the aphids were melon aphids in 1961 while only 19 percent were green peach aphids. No other aphid was collected on the "sticky boards" in significant numbers.

These data confirm a belief (1, 7) that virus diseases of cantaloups, particularly mosaic diseases, play a large role in the "crown blight complex" in Arizona.

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CONTROL OF ALTERNARIA BLIGHT OF RAPE AND MUSTARD BY GRISEOFULVIN

Akhtar Husain and Ram Nath Thakur[1]

Abstract

A single spray of griseofulvin applied before inoculation caused considerable reduction of leaf spot on yellow mustard. All concentrations of the antibiotic, ranging from 50 to 500 ppm, were effective in reducing disease intensity but higher doses were more effective than lower doses. In addition to the reduction of number of leaf spots, the treatment also reduced the size of leaf spots.

Alternaria blight (Alternaria brassicae (Berk.) Sacc.) is one of the most serious diseases of rape and mustard in India. It is especially serious on yellow mustard (Brassica campestris var. yellow sarson), brown mustard (Brassica campestris var. brown sarson) and toria (Brassica campestris var. toria) where it causes serious losses due to reduction in yield and oil content of the seed. All cultivated varieties of these crops have been found to be highly susceptible to the disease and the only possibility of control lies in use of fungicides.

Griseofulvin, an antifugal antibiotic, has been found effective against several diseases (1, 2, 3, 4, 5). Because of the systemic nature of griseofulvin it was considered desirable to test its effectiveness against Alternaria blight of rape and mustard; the preliminary results obtained are reported in this paper.

MATERIALS AND METHODS

A highly susceptible variety of yellow mustard (T. 151) was used in this experiment. The seeds were planted in 10-inch clay pots and thinned out at two-leaf stage to leave five plants in each pot. When the plants were 8 weeks old they were sprayed with an aqueous solution of griseofulvin ranging in concentration from 50 to 500 ppm. Each treatment was replicated six times in a randomized block design and untreated plants were used as a check. The plants were inoculated with a highly pathogenic strain of Alternaria brassicae, 4 days after application of the antibiotic, by spraying a heavy spore suspension of the fungus on the plants. The pots were kept in a humidity tent for 48 hours after inoculation. The plants were rated for disease intensity 4 weeks after inoculation; disease intensity was estimated by counting the number of spots on five lowermost leaves of each of the five plants in each replication. The final disease index was represented as average number of spots per leaf.

RESULTS AND DISCUSSION

The results are presented in Table 1. All concentrations of griseofulvin significantly reduced the number of leaf spots in comparison with the control. There were also significant differences among various treatments and 500 ppm proved to be most effective in suppressing the disease. At this concentration the antibiotic did not produce any phytotoxic effect on mustard. In addition to reduction in number of leaf spots, the treatment also checked the growth of spots. There was considerable injury in the check plants, resulting in complete drying and death of leaves, whereas all leaves of the plants treated with higher concentrations of the antibiotic remained green and had no chlorosis. The disease spots on the plant treated with 250 ppm and 500 ppm of griseofulvin consisted of only minute flecks without chlorotic halos and with no evidence of injury to leaves.

These observations indicate that griseofulvin, because of its systemic nature, may be efficient in controlling Alternaria blight on mustard leaves. The reduction in size of leaf spots indicates that the antibiotic retarded growth of the fungus even after it had entered the host.

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Table 1. Effect of griseofulvin on Alternaria blight of mustard.

Treatment (ppm)	Average number of leaf spots
50	31.3
100	23.0
250	11.3
500	5.2
Control	48.2

LSD .05 = 5.8

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A COMPARISON OF ISOLATION METHODS FOR FOMES ANNOSUS

Floyd F. Hendrix, Jr. and E. George Kuhlman[1]

Abstract

The number of samples and amount of wood colonized by Fomes annosus was compared by plating chips on agar media and by incubation in moist paper at 60° and 70° F. The number of samples infected and the depth of penetration into wood can be determined more accurately by plating than by incubating in moist paper at either 60° or 70° and examining the surface for conidia.

INTRODUCTION

Fomes annosus (Fr.) Cke. is a serious pathogen on many conifers throughout the world, causing root and butt rot as well as mortality (1, 2, 6). Because of the pathogen's economic importance, much research has been devoted to both the fungus and the disease. Many basidiomycetes grow slowly in culture, and plates are frequently overrun by faster growing fungi and bacteria when isolations are attempted. Several different techniques and media have been suggested for isolation of this fungus, and for determining the amount of wood colonized (4, 5, 7, 8, 9).

Wrapping wood or bark in wet paper and incubating it in a moist chamber at 15° to 20° C for 7 to 14 days has been suggested by Rishbeth (7, 8) as a technique for determining the amount of wood colonized. After incubation, the wood is examined for the presence of the conidial heads of the imperfect stage (Oedocephalum lineatum Bakshi). This technique is based on the assumption that F. annosus sporulates only on wood which has been colonized prior to incubation, and that it will sporulate on wood in which the fungus is viable.

Different media, including malt agar, potato-dextrose agar, and selective agar media, have been used for isolation of F. annosus from plant tissues (4, 5, 9). Several of these media, with various combinations of antibiotics and other fungistatic or fungicidal compounds, were tested by the authors to develop a selective medium for F. annosus. The medium described by Kuhlman and Hendrix (4) was the most satisfactory from the standpoint of inhibiting growth of bacteria and fungi other than F. annosus. Chip isolation techniques utilizing this agar medium were compared with wrapping as methods for determining the extent of colonization of wood by F. annosus.

MATERIALS AND METHODS

Ninety stumps of Pinus echinata were inoculated by spraying a conidiospore or basidiospore suspension of F. annosus on the freshly cut surfaces. Samples were collected by cutting into the stump at ground level for approximately 3 inches, and splitting off slabs 12 to 14 inches long. The slabs were submerged in a 0.5% aqueous solution of sodium hypochlorite (10% Clorox) for 10 minutes, then split vertically from the bottom into two sections. Small chips of wood were removed with sterile chisel-forceps and placed on the selective medium in Petri dishes (3). Six chips were taken horizontally across each section at each of the following levels: 1, 2, 3, and 5 inches from the top of the section, and 1 inch from the bottom. The six chips from each level were placed in separate dishes. In those sections in which the fungus had penetrated more than 5 inches down from the stump surface, additional cultures were made at 1-inch intervals until the depth of penetration was determined.

After removal of chips for plating, each section was wrapped in newspaper and wet thoroughly. The wrapped sections were incubated under polyethylene covers, half being held at 60° F and half at 70°. After 72 hours both surfaces of each of the sections were examined with a dissecting microscope at 20X magnification. The extent of spread downward, as indicated by sporulation on the wood, was measured. Measurements were recorded using the same depth classes previously established for the plating technique; that is, penetration to 1 but not 2 inches, and so forth. Thus, penetration as determined by direct isolation could be compared with that by the incubation technique.

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RESULTS

The average penetration of F. annosus in the 90 stumps 3 months after inoculation (Table 1) was 4.1 inches as determined by direct isolation. When the same stump sections were incubated in wet paper for 72 hours at 60° and 70° F, the average depth of penetration was 3.0 and 5.3 inches, respectively. These differences were statistically significant beyond the 0.01 level. Thus, three methods applied to the same material gave three different results. And these results varied by approximately 25% of the total penetration as determined by standard plating technique.

Table 1. Penetration of Fomes annosus into stumps of Pinus echinata as measured by plating and incubation in a moist chamber at 60° and 70° F for 72 hours.

Method of treatment	Depth of penetration into stumps (in inches)											Mean depth of penetration (inches)
	0	1	2	3	5	6	7	8	9	10	11	
	Number of stumps penetrated to each depth											
Plating	3	5	10	24	20	16	7	4	-	-	-	4.1
Incubation at 70° F	1	2	6	21	16	11	13	8	5	2	1	5.3
Incubation at 60° F	18	5	8	23	11	12	1	2	-	-	-	3.0

When the stump sections were incubated at 60° F, only 25% were penetrated to the same depth level determined by plating. In 40% of the sections the depth of penetration varied 1 inch or more from that determined by plating.

When wrapped stump samples were incubated at 70° F, F. annosus sporulated at a greater depth than was determined by the plating technique. The same depth of penetration was indicated by both methods in only 29% of the stumps. Penetration as measured by the wrapping technique varied from plating by 1 inch in 30% of the stumps and 2 or more inches in 41%, the largest amount of variation being 7 inches.

Similar comparisons were made with 500 stumps. Sections from these stumps were incubated at 60° F in the manner described previously, following isolation with the plating technique. F. annosus was isolated from 406 of 500 stumps by the plating technique. In 15% of these, however, it did not sporulate when split stump sections were held in moist paper at 60° for 72 hours. F. annosus sporulated on only 7 of 94 stumps that failed to yield the fungus by the plating technique.

DISCUSSION

The present study indicates that the wrapping technique will not reveal the location of F. annosus in wood or the amount of wood colonized as accurately as will the chip isolation method. The depths of penetration indicated by plating and by wrapping at both 60° and 70° F were significantly different statistically. The results of the wrapped readings at these two temperatures were also significantly different from each other. F. annosus can grow considerable distances over the surface of sound wood adjacent to colonized wood in 7 to 14 days under warm, moist conditions; it may grow as much as 7 inches in only 72 hours. After an incubation of 72 hours, the average depth of penetration at both temperatures varied an inch, or one-fourth of the mean penetration depth, from the plating technique. Spores of the fungus germinate and grow along the surface after only 3 days. Thus, the wrapping technique should not be used when precise determinations of the progress of this fungus through wood or the amount of wood colonized is desired.

When stump sections were wrapped in wet paper at 70° for 72 hours in some cases F. annosus grew and sporulated more abundantly on the paper than it did on the wood and grew from one sample to another through the paper if the samples were touching each other. In one experiment, plating detected the presence of F. annosus in 15% more stumps than wrapping. Conversely, in only 1% of the stumps tested was F. annosus detected by the wrapping technique but not by plating. With the selective medium described by Kuhlman and Hendrix (4), F. annosus was recovered by plating from 99% of wood samples in which the fungus was present. Thus, both the number of samples and the amount of wood colonized can be determined much more accurately by direct isolation than by the wrapping technique.

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OAK ANTHRACNOSE IN MISSISSIPPI

G. K. Parris[1] and Johnnie Byrd[2]

Abstract

A leaf spotting of certain Quercus spp. on the campus of Mississippi State University is reported. The causal agent is tentatively identified as Gloeosporium quercinum West. Symptoms vary from extensive individual spotting, terminal necrosis, twisting, brittleness of lamina, a type of shot-hole, to abscission. Certain species of Quercus are highly resistant. No studies of the sexual stage have been made so far, and the relationship of the local pathogen to sycamore anthracnose is largely unknown.

INTRODUCTION

According to Weiss (10) the genus Quercus is attacked by the disease called anthracnose due to Gloeosporium spp. The Gloeosporia listed are:

1) G. bicolor J. J. Davis (G. bicolor McAlp. has priority) on Q. bicolor (swamp white oak) in Wisconsin.

2) G. canadense Ell. & Ev. (= G. quercinum West. ?) on Q. alba (white oak), Q. bicolor, and Q. macrocarpa (burr oak) in Connecticut, Kentucky, Iowa, Michigan, New York, and Wisconsin.

3) G. divergens Pk. (= G. quercinum West. ?) on Q. alba in New York.

4) G. martini Sacc. & Ell., the asexual stage of Marssonina martini (Sacc. & Ell.) Magn., the cause of brown-bordered leaf spot or eyespot. General on white oaks (Q. alba, Q. bicolor, Q. lyrata, Q. macrocarpa and Q. stellata) from New York to Mississippi, Texas, and North Dakota.

5) G. nervisequum (Fckl.) Sacc. (a synonym of G. quercinum) on white oaks.

6) G. quercinum West. This is the asexual stage of Gnomonia quercina Kleb., cause of anthracnose, leaf and twig blight. On Q. alba in Massachusetts to Georgia, Kansas, and Minnesota, on Q. bicolor in New York, on Q. macrocarpa in Kentucky to Kansas and Wisconsin, and on Q. stellata in Oregon and possibly in Georgia. Weiss says of this form, "Has usually been identified as Gnomonia veneta (Sacc. & Speg.) Kleb., an untenable name even when applied to the anthracnose fungus on Platanus. As the oak fungus differs in some respects it seems best to designate it by a distinctive name." Weiss does not list Gnomonia veneta on Quercus.

Gnomonia quercina (Gloeosporium quercinum) is also recorded by Weiss on Q. garryana (Oregon white oak) in that State; on Q. montana (chestnut oak) and Q. prinus (swamp chestnut oak) in Connecticut, New Jersey, and West Virginia; as general on members of the red, black, and pin oak group, namely Q. borealis (northern red oak), Q. coccinea (scarlet oak), Q. ellipsoidalis (northern pin oak), Q. falcata (southern red oak), Q. palustris (pin oak), and Q. velutina (black oak) in eastern and central States, also California and Oregon; on Q. agrifolia (California live oak) in that State and Oregon; and on Q. virginiana (southern live oak) in Mississippi.

Parris (6) in his revision of Presley's 1947 Host Index of Mississippi Plant Diseases lists Gloeosporium quercinum on Q. rubra, G. sp. on Q. alba, and Gnomonia quercina on Q. virginiana. The brown-bordered leaf spot fungus Marssonina martini is listed by Parris on Q. alba, Q. macrocarpa, Q. montana, Q. muhlenbergii (yellow oak), Q. prinoides (dwarf chinquapin oak), and Q. prinus.

Turning now to the sycamore, Weiss (10) lists Gnomonia veneta (Sacc. & Speg.) Kleb. (= Gloeosporium nervisequum (Fckl.) Sacc., Gnomonia platani Edg.) as causing anthracnose, twig blight, and canker on Platanus acerifolia (London planetree) in California, New Jersey and New York, as general on P. occidentalis (American planetree or sycamore) and P. orientalis (Oriental planetree), and in California on P. racemosa (California sycamore or planetree). Parris (6) lists Gnomonia veneta on P. occidentalis but not on oak.

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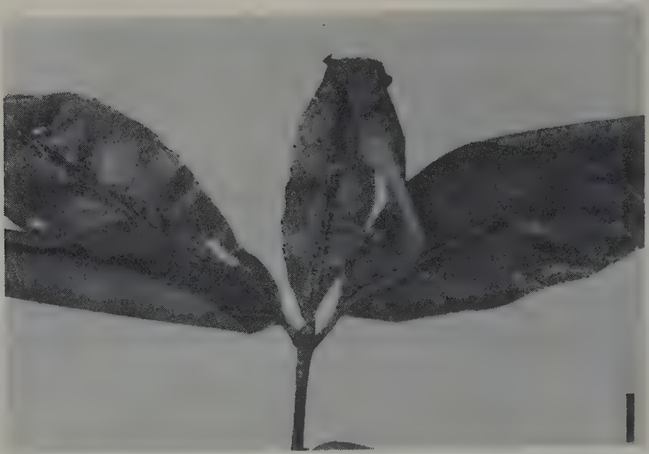

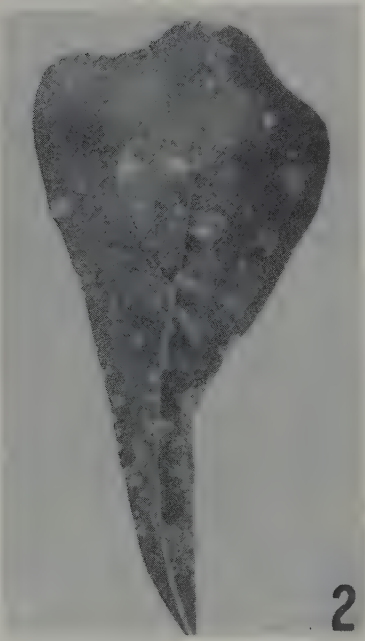

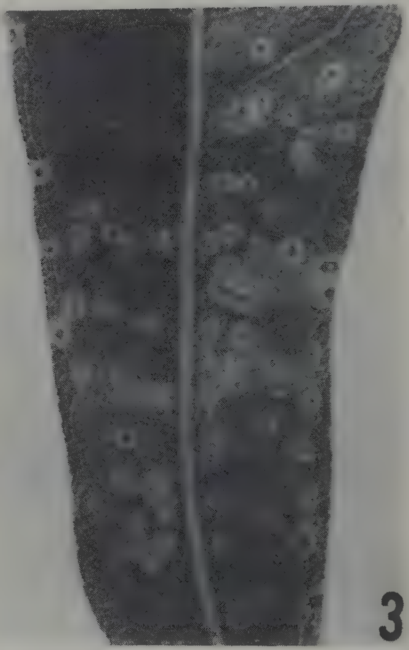

FIGURE 1. Anthracnose on willow oak leaf.
FIGURE 2. Anthracnose on water oak leaf.
FIGURE 3. Anthracnose on water oak; closeup of Figure 2.

SYMPTOMATOLOGY

A leaf spot, which could be called anthracnose, or blight in extreme cases, sometimes causing a type of shot-hole, accompanied by considerable abscission has been noticed on the Quercus species on the campus of Mississippi State University since 1956 or thereabouts. When first observed, it was not serious, and its cause was casually attributed to the feeding punctures of some unidentified insect. Subsequently, the disease has become serious, for it can cause defoliation preceded by the above-mentioned spotting over much of the surface of a leaf.

Typically, especially on Q. phellos (willow oak) and Q. laurifolia (laurel oak), the spots are numerous, tiny, dark brown or black, with healthy tissue surrounding them (Fig. 1)[3]. On Q. nigra (water oak) and Q. falcata var. pagodaefolia (cherrybark oak), the spots are larger, lighter in color, and tend to be surrounded by yellowed areas (Figs. 2 and 3). In cases of severe attack, the tips of affected leaves wither and die, curl, develop a type of shot-hole especially on Q. palustris (described below), and dehisce in varying degrees according to species of

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[3] All photos by Norman Merwine.

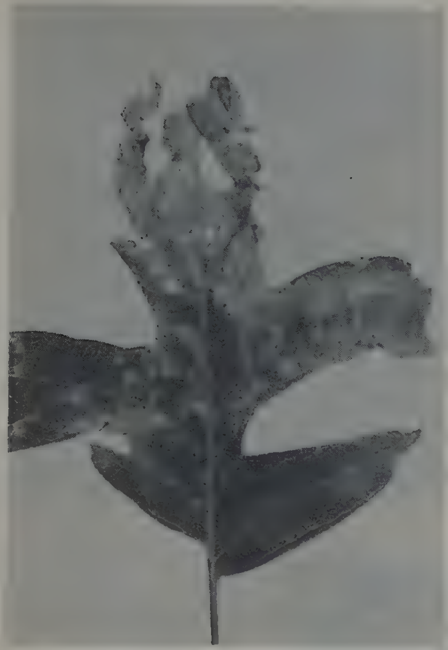


FIGURE 4. Anthracnose on pin oak leaf, showing shot-hole and brittleness of lamina.

Quercus attacked. Most severely affected species are Q. phellos, Q. nigra and Q. laurifolia. Also attacked, but less severely -- primarily because the diseased areas, pinpoint or two to three times larger in extent, fall away in a shot-hole effect (Figs. 4 and 5) -- is Q. palustris (pin oak) and Q. shumardi (shumard oak). On these species, lesions are on the upper surface mostly, at first approximately circular, dark-reddish brown, pinpoint-sized to two to three times this size, later becoming shot-holed, the hole surrounded by a hydrotic, paler green than normal to light yellow margin. The leaf spots as they shothole become depressed toward the lower surface, and in final aspect this surface shows sharp points while the upper surface shows tiny funnels, through which light shows. Spotting on the lamina seemingly is at random, does not occur on the veins, though these structures are touched by necrosis but not severed. Accompanying the shot-hole can be a general laminal puckering, especially if the attack came while the leaf was 1/3 to 1/2 grown; a brittleness is associated with the necrosis and this gives an extremely ragged looking pin oak or shumard oak leaf (Fig. 6).

Quercus falcata var. pagodaefolia and Q. virginiana are little affected by anthracnose, seldom showing lesions, or at most less than half a dozen inconspicuous ones on a leaf. In contrast, a susceptible Quercus can show as many as 100 to 150 lesions per square inch of leaf surface. Quercus durandii shows possibly a few more spots than Q. virginiana, with damage almost nil, but data on this species are less complete. Quercus stellata (Fig. 7) and Q. falcata var. falcata seem to be extremely resistant; one hesitates to use the term immune.

When abscission occurs, a set of new leaves appears, which is attacked if weather conditions (rainy windy conditions) are favorable. This is in contrast to the effect of Taphrina coerulescens on Quercus spp., where new growth usually remains fairly free from attack following loss of the first leaves due to the leaf blister fungus.

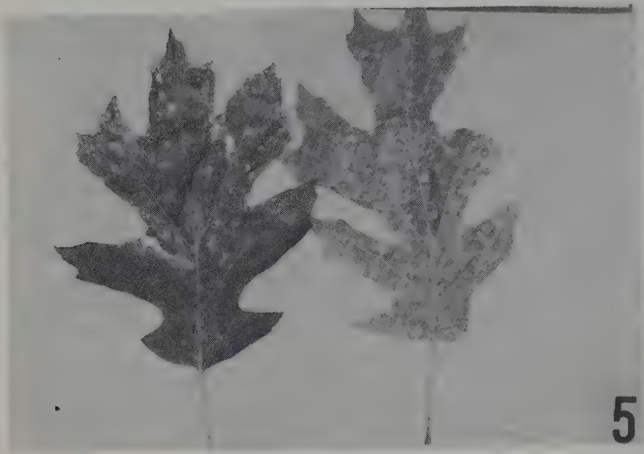
There are no sycamore trees on the campus at Mississippi State University near the diseased oaks, and to the present no search has been made for anthracnose on Platanus spp.; however, such a search is planned.

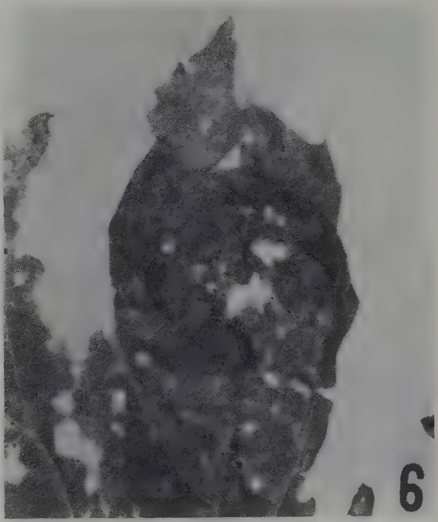
ETIOLOGY

Cultures from this leaf spot have yielded a slow-growing fungus which forms its conidia in acervuli, which in general correspond to Gloeosporium nervisequum (Gnomonia veneta) as diagrammed by Barnett (1) (p. 201, Fig. 283 A-F). Good material has also been obtained by placing fragments of diseased Quercus leaf on a glass slide in a drop of water, covering with a cover slip, and placing the slide in a moist chamber for 3 to 4 days at room temperature (75° to 85° F). The acervuli develop on the lesions, fruit vigorously, and hyphae ramify out of the host tissue into the surrounding water medium. No measurements of conidia have been made.

What fungus are we dealing with here? As recently as April 1962, Hepting and Fowler (5) illustrated what they designate as "anthracnose on white oak" which looks very much like the Mississippi State disease on local susceptible species. We have no white oaks on the campus, and on this oak the disease has not been studied in detail, though it exists. Hepting and Fowler's bulletin carries the following sentence, "Hardwood leaf diseases seldom kill much tissue until seasonal growth is complete, and even such conspicuous diseases as elm leaf spot and leaf blotch of horse chestnut seldom interfere seriously with manufacture of reserve food. Notable exceptions are sycamore and oak anthracnose (Fig. 1 B) . . . . and a few others that hit so hard and so early they disfigure leaves and can check growth and lead to death if attacks are repeated." These workers speak of the oak and sycamore pathogen as if it were the same.

There is almost nothing in the literature describing G. quercinum on oak. Fergus (3) writes about an epiphytotic of white oak anthracnose in central Pennsylvania; Yarwood (9)





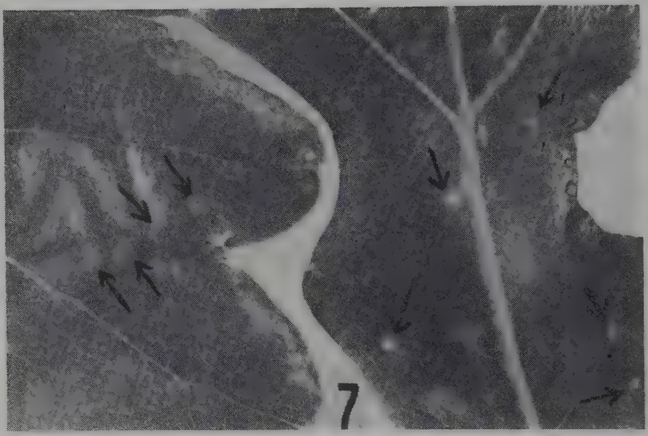


FIGURE 5. Anthracnose on shumard oak leaf, upper surface on left, lower surface on right. Perforations (shot-hole) are especially evident, also the ragged aspect due to brittleness of necrosed areas.

FIGURE 6. A closeup view of effect of anthracnose on tip of a shumard oak leaf.

FIGURE 7. Arrows indicate effect of anthracnose on the highly resistant post oak leaf; a sort of local lesion response.

discusses defoliation of wild and cultivated sycamore by the imperfect stages of Gnomonia veneta during rainy weather; and Fergus (4) in a later paper acknowledges Weiss' (10) designation of the oak anthracnose pathogen as differing in some respects from the sycamore anthracnose pathogen, and his (10) preference for the name of the oak pathogen as Gloeosporium quercinum (Gnomonia quercina), but continues to refer to the white oak anthracnose pathogen as Gnomonia veneta Kleb. Fergus (4) mentions a shattering of the brittle necrotic leaf tissue by the oak disease. This fits the disease as it appears on the Mississippi State University campus on willow oak, shumard oak, and sometimes on pin oak.

Schuldt (7) studied anthracnose fungi he cultured from infected oaks and plane trees in Iowa, but did not describe the diseases they caused. He found that these fungi fell into two well-defined groups, a Platanus group which included conidial isolates from plane, white oak (Q. alba) and burr oak (Q. macrocarpa) and also perithecial stages on overwintered leaves of white oak and plane, all of which he included in Gnomonia veneta; and a Quercus group which included conidial isolates from red oak (Q. borealis), white oak, American elm, and black walnut. Conidia of the oak group type were never obtained from single ascospore cultures.

Isolates of the plane type from plane and isolates of the oak type from white oak were both able to infect white oak and plane. Schuldt proposed that the white oak group be referred to Gloeosporium quercinum until proof of their identity is more definitely established, though there was some evidence that this group is closely related to Gnomonia veneta.

Stoneman (8) described the effects of Gloeosporium nervisequum on oak and sycamore, namely, as shrunken withered veins bordered by arid brown patches. The fungus caused the leaves to curl and become much distorted. Barnett (1) pictures this fungus on the veins of Platanus with some adjacent discoloration of the blade.

Boyce (2) writes about leaf and twig blight of sycamore and oak, which he attributes to Gnomonia veneta. "Sycamores particularly and to some extent oaks are attacked. On oaks the disease is usually on leaves, though twigs may be affected, but on sycamores both leaves and twigs are attacked .... (the asexual stage) is known as Gloeosporium nervisequum. Killed leaves soon drop ...." Boyce's writings are mostly of the effect of the disease on sycamore veins and what he describes is not what is now present on Quercus leaves on the Mississippi State University campus.

The fungus we are reporting here definitely does not attack the veins and would therefore seem not to be G. nervisequum (= Gnomonia veneta). We are tentatively calling it G. quercinum West. until further studies, particularly a search for the sexual stage, and also the presence of anthracnose on sycamore, can be completed.

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UNUSUAL SEVERITY OF LEAF-BLISTER ON CERTAIN SPECIES OF OAK IN MISSISSIPPI

G. K. Parris[1]

In the spring of every year oak leaf blister, Taphrina coerulescens (Mont. & Desm.) Tul., occurs on the campus of Mississippi State University, where there is a rather unique collection of Quercus spp. Several masters' theses have been worked here on the diseases of this genus.

This paper records such an extremely heavy infection of leaf-blister at State College, especially on Q. falcata var. pagodaefolia (cherrybark oak), as to suggest that the upper surfaces of diseased leaves had been painted a light yellowish-white (Fig. 1). Water oak, laurel oak, live oak, and willow oak also are being severely attacked.

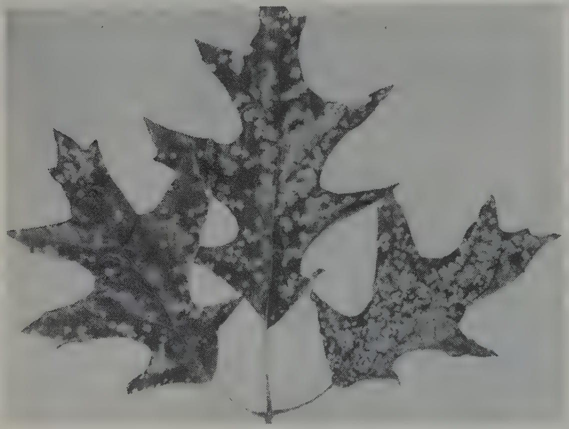

FIGURE 1. Extensive growth of Taphrina coerulescens on cherrybark oak leaves. (Photograph by Norman Merwine)

In 1953-54, former graduate assistant in botany at Mississippi State Dr. M. J. Goode, while working on his M. S. degree[2], classified the oaks on the campus as highly resistant, slightly susceptible, or highly susceptible to T. coerulescens: post oak (Quercus stellata), pin oak (Q. palustris), shumard oak (Q. shumardi), and chinquapin oak (Q. muhlenbergi) were highly resistant; blackjack oak (Q. marilandica), southern red oak (Q. falcata), and black oak (Q. velutina) were slightly susceptible; water oak (Q. nigra), laurel oak (Q. laurifolia), live oak (Q. virginiana), and willow oak (Q. phellos) were highly susceptible. The members of the last group dehisce their leaves easily when attacked, and a new set may appear during the summer. The second set of leaves subsequently shows no leaf-blister, or very little.

At the time when Dr. Goode was doing his research on Taphrina, the three specimens of Quercus falcata on the campus had not been fully recognized as consisting of two subspecies, Q. falcata var. falcata (southern red oak) and Q. falcata var. pagodaefolia. Goode's report that Q. falcata is slightly susceptible fits the usual response of the single specimen of Q. falcata var. falcata on the campus. One of the two local specimens of Q. falcata var. pagodaefolia showed extreme susceptibility (Fig. 1) while the other was highly resistant, with blistering infrequent or, when present, insignificant and limited to a small amount of yellowing with little reproduction by the fungus.

Taxonomic studies are planned to clarify the Quercus falcata complex on the campus; there is a distinct possibility that we are dealing with hybrids within the two subspecies. In this research, inoculation with the oak leaf-blister fungus will be used as an aid to proper identification of the host.

Anthracnose, tentatively considered to be caused by Gloeosporium quercinum West. and first observed about 4 years ago at Mississippi State, for the second successive year is taking a heavy toll of leaves of water oak, willow oak, and laurel oak; this will be discussed more fully in a separate article to follow.

DEPARTMENT OF BOTANY, MISSISSIPPI STATE UNIVERSITY,
STATE COLLEGE, MISSISSIPPI

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[1] Head, Botany Department, Mississippi State University, State College, Mississippi.

[2] Goode, Munroe Jack. 1954. Studies on the life cycle and control of Taphrina coerulescens (Mont. & Desm.) Tul. M. S. thesis, Mississippi State University, January, 1954.

NEW HOSTS AND NONHOSTS OF RENIFORM NEMATODE[1]

Wray Birchfield and L. R. Brister[2]

Summary

Forty three agronomic plants were tested for their reaction to reniform nematode. Eleven nonhost plants were discovered that are adaptive to growing in Louisiana where this organism is a serious plant parasite. These plants are: barnyard grass, dallis grass, mustard, oats, onion, pepper of two species, rice, sorghum, sugarcane, turnip, and wild barley. Six new host plants, all legumes, were found. These are: crimson clover, red clover, white clover, hairy vetch, singletary pea and peanuts.

INTRODUCTION

The reniform nematode, Rotylenchulus reniformis Linford & Oliveira, 1940, characteristically parasitizes a wide variety of plant life in broad taxonomic groups. The first host plant work on this organism was that of Linford and Yap in 1940 (1) in Hawaii. Only a few of the 65 plants tested by these workers showed a high degree of resistance and complete immunity was not indicated. Smith and Taylor (10) reported it in the United States from cotton and soybeans in Louisiana in 1941. It was found again in Louisiana in 1952 by Martin and Birchfield (5) on cotton and in 1960 by Martin (4) on sweetpotato. Peacock (7) reported several hosts from the African Gold Coast and Martin (3) reported other host plants in South Africa, while Luc and Guiran (2) listed many additional hosts from West Africa. Steiner (11) observed reniform nematode on tomato and coffee weed from Quincy, Florida. Van Weerdt, et al. (13) also encountered it on several ornamentals in Florida, as well as a new species of the genus associated with a severe disease of coconut palm. It is known to occur in Alabama on cotton (6) according to Minton and Hopper and also in Georgia (9) according to Smith. Thorne (12) observed the pest infecting tobacco, tea, clove and other plants in Java and clove in Sumatra. More recently reniform nematode was reported from South America for the first time by Sasser, et al. (8) abundantly associated with cotton roots in Peru. The authors observed reniform nematode in sugarcane soil from the Dominican Republic.

In Louisiana this organism is generally considered a severe plant parasite of cotton, involving a large amount of acreage. Need for information on nonhost plants of the reniform for use in crop rotation prompted an investigation for search of such hosts. Of particular importance in crop rotation in Louisiana are certain grasses and legumes of which little was known of their susceptibility. These were tested to acquire this information.

MATERIALS AND METHODS

Test plants were in most cases propagated from seed in 8- or 12-inch clay pots of naturally infested field soil placed in the greenhouse. The population of reniform nematode averaged 300 to 400 per pint of soil. The appearance of egg masses on the roots was used to indicate the host or nonhost preference of the organism. The number of egg masses on individual plants was employed as a criterion to assign the degree of resistance expressed by plant species. When plants were not obviously infested with egg masses and/or swollen females, they were overstained in cold lactophenol-acid fuchsin. The animals absorb the red stain, while all plant tissues except xylem and damaged cells remained unstained. Tests plants were classed according to number of egg masses: 0 = immune, 0-5 = resistant, 5-25 = moderately resistant, and 25-more egg masses = susceptible. Plants were examined at approximately 30-, 62-, and 120-day intervals. Susceptible plants were generally detected after 30 days, but nonhost plants were more difficult to determine, necessitating three different examinations and bioassay with susceptible tomato to determine whether the parasite remained alive.

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[1] Cooperative investigations with the Department of Botany and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana.

[2] Nematologist and Agricultural Aid, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

RESULTS

Test plants employed and the degree of resistance of these plants are shown in Table 1.

Table 1. Host-plant reactions to the reniform nematode.

Common name	Scientific name	No. egg masses[a]	Degree of resistance
Barley, wild	*Hordeum pusillum*	0	Immune
Barnyard grass	*Echinochloa crus-galli*	0	Immune
Bean, bush, Tennessee Greenpod	*Phaseolus vulgaris* var. *humilis*	25-more	Susceptible
Bean, bush lima, Henderson	*Phaseolus limensis* var. *limenanus*	25-more	Susceptible
Beet, dark red, Detroit	*Beta vulgaris*	25-more	Moderately resistant
Bermuda grass, common	*Cynodon dactylon*	0	Immune
Broccoli, Waltham 29	*Brassica oleracea*	0-5	Resistant
Carrot, Scarlet Nanthes	*Daucus carota* var. *sativa*	0-5	Resistant
Cauliflower, Snowball	*Brassica oleracea* var. *botrytis*	0-5	Resistant
Clover, crimson	*Trifolium incarnatum*	25-more	Susceptible
Clover, white	*Trifolium repens*	25-more	Susceptible
Clover, tensas	*Trifolium* sp.	25-more	Susceptible
Corn, Golden Cross	*Zea mays* var. *rugosa*	0-5	Resistant
Corn, Golden Crown	do.	0-5	Resistant
Corn, Golden Bantam	do.	0-5	Resistant
Corn, Stowell's Evergreen	do.	0-5	Resistant
Corn, Ivana Single Cross	do.	0-5	Resistant
Corn, Country Gent	do.	0-5	Resistant
Corn, Trucker's	*Zea mays* var. *indentata*	0-5	Resistant
Cotton, DPL-Smoothleaf	*Gossypium hirsutum*	25-more	Susceptible
Cucumber, Improved Long Green	*Cucumis sativus*	25-more	Susceptible
Dallis grass	*Paspalum dilatatum*	0	Immune
Lettuce, Iceburg	*Lactuca sativa*	5-25	Moderately resistant
Marigolds, Harmony mix	*Tagetes erecta*	0-5	Resistant
Muskmelon, New Orleans	*Cucumis melo*	25-more	Susceptible
Mustard, Florida	*Brassica nigra*	0	Immune
Oats, fulghum	*Avena sativa*	0	Immune
Okra, Louisiana Market	*Hibiscus esculentus*	0-5	Resistant
Onion, Evergreen	*Allium cepa*	0	Immune
Peanuts, Spanish	*Arachis hypogaea*	0-5	Resistant
Peas, singletary	*Lathyrus hirsutus*	25-more	Susceptible
Peas, Purple Hull	*Vigna sinensis*	25-more	Susceptible
Pepper, Sweet Bell, California Wonder	*Capsicum annuum*	0	Immune
Pepper, red, hot	*Capsicum annuum* var. *fasciculatum*	0	Immune
Pumpkin, Greenstriped Cushaw	*Cucurbita pepo*	25-more	Susceptible
Rice, Blue Bonnet	*Oryza sativa*	0	Immune
Sorghum, sweet	*Sorghum vulgare*	0	Immune
Spinach, Bloomdale, Savoy	*Spinacia oleracea*	0	Immune
Squash, Early White Bush, Scallop, Calabaza	*Cucurbita pepo* var. *melopepo*	25-more	Susceptible
Sugarcane, C. P. 44-101	*Saccharum officinarum*	0	Immune
Turnip, Purple Top, White Globe	*Brassica rapa*	0	Immune
Watermelon, Stone Mountain	*Citrullus vulgaris*	25-more	Susceptible
Vetch, hairy	*Vicia villosa*	25-more	Susceptible

[a] Egg masses based on average per root system.

DISCUSSION

All grasses tested, except corn, were immune from reniform nematode in that reproduction of the parasite did not occur on these plants. Furthermore, penetration by young females of the immune plants was not observed in root tissue stained with acid fuchsin. All corn varieties tested were resistant to egg production of the parasite in that few egg masses on these plants were observed in contrast to the legumes tested which were susceptible with numerous egg masses produced on them.

According to the host plants and degrees of resistance to the reniform nematode of Hawaii as reported by Linford and Yap (1), the Louisiana population is believed to differ physiologically; however, the morphology is closely similar. Also a population from the Dominican Republic associated with sugarcane is morphologically similar to the Louisiana population which failed to parasitize sugarcane. It is suggested that different strains or species occur among the presently conceived reniform nematode.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BATON ROUGE, LOUISIANA

INTRACELLULAR INCLUSIONS OBSERVED IN EPIDERMAL STRIPS FROM FOLIAGE OF CADANG-CADANG INFECTED COCONUT TREES IN THE PHILIPPINES

D. B. Protacio

Many plant virus diseases are characterized by intracellular inclusion bodies. The association of such inclusions with virus diseases has been reported in mammals, birds, insects (3), as well as in plants (2, 5). It was therefore decided to make a study of leaves from coconut palms infected with cadang-cadang, to see whether inclusion bodies were associated with this disease. This decision was prompted by existing evidence concerning the virus etiology of the cadang-cadang disease, as Price (4) said: "The evidence in favor of the virus hypothesis is very strong whereas the evidence against it is very weak."

Epidermal strips of diseased leaves from 23 trees were examined for the presence or absence of intracellular inclusions, and in particular for so-called "X-bodies," an amoeboid type of inclusion characteristic for many plant virus diseases. Epidermal strips were fixed in Bald's iodine-potassium iodide formalin solution (1) and were stained with Giemsa stain. Spherical bodies, intracellularly situated, were observed in all but one of the 23 trees (Fig. 1B and 2). These dark-blue stained bodies were found to resist immersion in 50 to 100% ethyl alcohol for an hour and boiling in water for the same length of time. Bodies in strips previously examined and subjected to autoclave pressure of 15 pounds per square inch for 15 minutes showed no appreciable damage. They remained intact in plain distilled water and 50% ethyl alcohol even after 52 days of storage.

An examination of all leaves in the crown of trees with varying degrees of infection revealed that the two youngest leaves, which were without external symptoms, did not exhibit spherical bodies. Very few inclusions were present in the third leaf. All the older leaves in the crown exhibited these bodies.

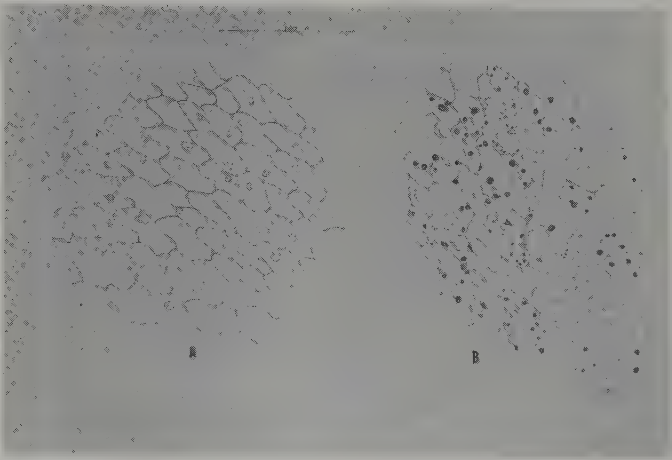

FIGURE 1. Camera lucida drawings of epidermal strips from foliage of coconut. A -- Group of cells from healthy epidermal strips showing spherical nuclei. B -- Group of cells from cadang-cadang diseased epidermal strips showing spherical intracellular inclusion bodies. Nuclei tending to elongate assume spindle shape. (Photograph by J. Nuñez.)

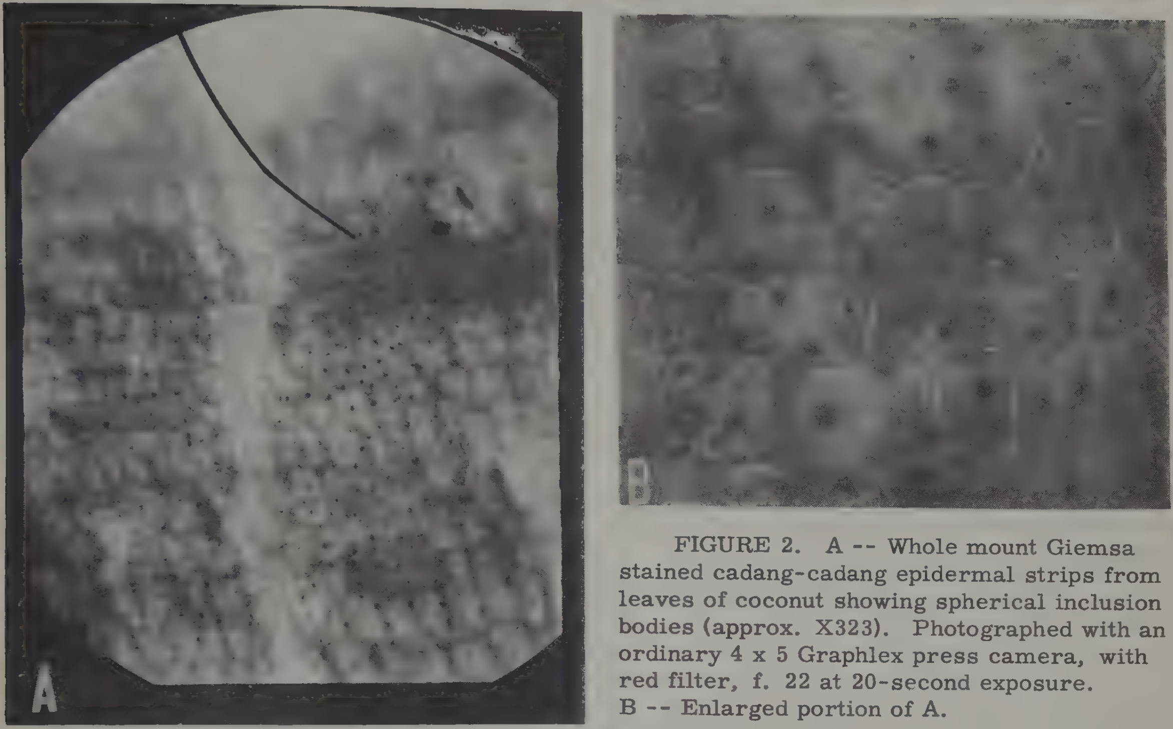

FIGURE 2. A -- Whole mount Giemsa stained cadang-cadang epidermal strips from leaves of coconut showing spherical inclusion bodies (approx. X323). Photographed with an ordinary 4 x 5 Graphlex press camera, with red filter, f. 22 at 20-second exposure. B -- Enlarged portion of A.

The healthy strips, kindly furnished by Mr. Celestino T. Rivera, Senior Entomologist of this Station, came from five coconut trees in Batangas Province region, outside the cadang-cadang disease area. No abnormal inclusion bodies similar to those found among cadang-cadang infected trees were seen in the Batangas material. Moreover, only 1 of 13 apparently healthy trees in the immediate vicinity of the Guinobatan Experiment Station, within the cadang-cadang disease area, exhibited similar bodies.

Recently additional epidermal strips from five apparently healthy trees and from five trees in various stages of cadang-cadang infection were stained, following the procedure described. The strips from the five apparently healthy trees did not show inclusion bodies, with one exception, while all the strips from diseased leaves showed these bodies. Observation of this peculiar abnormal inclusion offers an unusually favorable opportunity for a diagnostic test of the cadang-cadang disease.

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EFFICACY OF FALL APPLICATIONS OF COPPER AND ORGANIC FUNGICIDES FOR THE CONTROL OF CORYNEUM BLIGHT OF PEACH IN CALIFORNIA

Harley English and J. R. Davis[1]

Abstract

Spray tests conducted over a 3-year period have shown that late fall applications of ziram and ferbam are effective in the control of Coryneum blight of peach. These fungicides compared favorably with copper materials and were superior to captan, dichlone, dodine, and nabam tank mixed with iron and zinc sulfates. The efficacy of ziram and ferbam was not enhanced by the addition of adhesives, even though their residual life was somewhat extended. Fruit from sprayed trees contained only traces of the active ingredients. None of the materials tested appeared to be phytotoxic.

INTRODUCTION

Recent work in California (3) has shown that some of the dithiocarbamate fungicides, when used as fall sprays, provide effective control of peach leaf curl (Taphrina deformans (Berk.) Tul.). Since, however, the fall application is designed to control both leaf curl and Coryneum blight (Coryneum beijerinckii Oud.) large-scale adoption of these materials for use on peaches could not be made until research had demonstrated their effectiveness against the latter disease. The Coryneum blight fungus infects peach twigs in California during the wet weather of late fall, winter, and early spring. Effective control of such a disease requires, therefore, that a protective fungicide, if applied only once, have a long residual life. Bordeaux mixture, or fixed copper materials applied with an adhesive, have generally met this requirement and therefore have been the fungicides usually recommended. At the time the present experiments were begun, the authors were extremely skeptical that any of the organic materials would prove to be sufficiently stable and adhesive to enable them to function effectively as single fall sprays.

Also, when the present investigation was started relatively little research had been done to ascertain the effectiveness of the organic fungicides in the control of Coryneum blight of stone fruits. Preliminary work by Hoffman (5) in California indicated that some of the dithiocarbamates offered promise in controlling this disease on almond and peach, and Morschel (6) in Australia reported that thiram was effective as a fall and spring spray on apricot. More recently work in Argentina (7) showed that late fall sprays of ziram and ferbam aided in controlling Coryneum blight of peach, and research in Italy (4) indicated that fall, winter, and spring sprays of ziram were more effective than Bordeaux mixture in controlling this disease. Recent work in Oregon (1, 2) also indicates that some of the organic fungicides provide satisfactory control of peach Coryneum blight when applied as single fall sprays. In the present study, organic materials that had shown promise in controlling peach leaf curl (3) were compared with copper fungicides for the control of Coryneum blight of peach.

MATERIALS[2] AND METHODS

The fungicides used in the 1956 test were ziram 76% (Zerlate), ferbam 76% (Fermate), nabam 19% (Dithane D-14), tank mixed with iron and zinc sulfates, captan 50% (Orthocide 50W), dichlone 50% (Phygon XL), a combination of ziram 59.8% and zinc benzothiazylmercaptide 5.2% (Niacide Z), and Bordeaux mixture. An attempt was made to increase the residual life of most of the organic materials by the addition of either dormant oil emulsions or a proprietary adhesive (FS-55) manufactured by the Peerless Spray Chemical Company. The sprays were applied by hand gun at approximately 400 pounds' pressure to eight randomized, single-tree replicates of mature Peak peach trees in San Joaquin County. The applications were made November 28, 1956 and Coryneum blight twig lesions were recorded April 4, 1957. Three randomized unsprayed trees served as checks.

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[2] The cooperation of the following companies who supplied test materials is gratefully acknowledged: E. I. du Pont de Nemours and Company, Rohm and Haas Company, United States Rubber Company, California Chemical Company, Food Machinery and Chemical Corporation, American Cyanamid Company, and Peerless Spray Chemical Company.

The fungicides that appeared most promising in the 1956 test were again tested, with some modifications in concentration, in 1957. One of the fixed copper materials (Orthocop 53) also was included for comparison with Bordeaux mixture. The orchard, plot arrangement, and method of application were the same as in 1956. The sprays were applied November 22, 1957, and disease incidence was recorded May 19, 1958. Analyses of the active ingredient on trees sprayed with ziram and ferbam were made by collecting twig samples on the day of spraying and approximately 2 and 3 months later. The analytical work was done by E. I. du Pont de Nemours and Company.

The 1958 test was conducted in a peach orchard in Placer County using mature trees thought to be a sport of the variety Red Bird. The plot arrangement and method of application were essentially the same as in the two previous tests. The only new fungicide employed was dodine 70% (Cyprex). The sprays were applied November 21, 1958 and twig lesions were counted April 16, 1959. A similar test was conducted in the fall of 1959, but extremely low precipitation during the winter reduced the twig infection to such an extent that an evaluation of the treatments was impossible. However, fruit was collected from the ziram and ferbam plots the following summer for residue analyses by the Pesticide Residue Research Laboratory of the University of California. The colorimetric method developed by E. I. du Pont de Nemours and Company was used in these analyses. It is reported to have a sensitivity of 0.05 ppm.

RESULTS

As shown in Table 1, both ziram and Niacide Z were highly effective in reducing the number of twig lesions, and ferbam appeared to be almost as good. Nabam and captan were significantly poorer than ziram, and dichlone was the least effective of the materials tested. When an analysis was made of the data obtained with the more effective fungicides, ziram, ferbam, and Niacide Z were found to be superior to Bordeaux mixture at the 1% level of significance.

The results obtained in 1957 were complicated to some extent by the occurrence of bud killing due to the bacterial canker organism (Pseudomonas syringae van Hall) as well as C. beijerinckii. Since the bud-killing symptoms of these two diseases are very similar, some of the blight infections recorded in Table 2 were undoubtedly the result of infection by P. syringae. Despite this difficulty, ziram and Niacide Z compared favorably with Bordeaux mixture, and the results with ferbam were comparable to those obtained with Orthocop 53. It is of interest that although the adhesives increased the initial and final deposits of ziram and ferbam, this greater residue was not correlated with enhanced disease control. There is even some indication that the oil emulsion may actually have interfered slightly with the fungitoxic action of these materials. Precipitation in the vicinity of the orchard during the period from November 22, 1957 to February 27, 1958 was 9.77 inches, approximately twice the normal amount for this locality.

Table 1. Efficacy of Bordeaux mixture and several organic fungicides in the control of Coryneum blight of peach, San Joaquin County, 1956-57.

Treatment[a] (amount/100 gallons)	Twig lesions per replicate[b] (average number)
Ziram 3 lb	1.8
Ziram 3 lb + Peerless FS-55 Adhesive 3 pt	1.5
Ferbam 3 lb	11.1
Nabam 1 gal. + iron and zinc sulfates	108.8
Nabam 1 gal. + iron and zinc sulfates + Sunland Flowable Dormant Oil Emulsion 1 gal.	88.3
Captan 3 lb + Sunland Flowable Dormant Oil Emulsion 1 gal.	77.0
Dichlone 1 1/2 lb	169.8
Niacide Z 3 lb + Peerless FS-55 Adhesive 3 pt	2.9
Bordeaux mixture 10-10	34.0
Unsprayed	216.0[c]
LSD .05	74.3

[a] Sprays were applied on November 28, 1956 to eight randomized single-tree replicates.

[b] Lesions on 60 6-inch twigs per replicate were counted April 4, 1957.

[c] Excluded from statistical analysis because only three replicates employed.

Table 2. Efficacy of copper and dithiocarbamate fungicides in the control of Coryneum blight of peach, San Joaquin County, 1957-58.

Treatment[a] (amount/100 gallons)	Residue of active ingredient in ppm 11-22-57	1-20-58	2-27-58	Blight infections per replicate[b] (average number)
Ziram 2 lb	242	42	7	19.6
Ziram 2 lb + Peerless FS-55 Adhesive 2 pt	290	61	15	19.8
Ziram 2 lb + Ortho Kleenup Flowable Emulsion 1 1/2 gal.	356	82	33	25.4
Ziram 3 lb	324	78	13	20.8
Ferbam 3 lb	274	60	10	25.9
Ferbam 2 lb + Ortho Kleenup Flowable Emulsion 2 gal.	354	118	66	33.3
Niacide Z 3 lb	--	--	--	14.6
Niacide Z 3 lb + Peerless FS-55 Adhesive 3 pt	--	--	--	17.9
Orthocop 53 5 lb + Ortho Kleenup Flowable Emulsion 2 gal.	--	--	--	25.8
Bordeaux mixture 10-10	--	--	--	14.6
Unsprayed	--	--	--	73.0[c]
LSD .05				9.1

[a] Sprays were applied November 22, 1957 to eight randomized single-tree replicates.
[b] Twig lesions and blighted buds on 40 6-inch twigs per replicate were counted May 19, 1958.
[c] Excluded from statistical analysis because only three replicates employed.

Table 3. Efficacy of copper and several organic fungicides in the control of Coryneum blight of peach, Placer County, 1958-59.

Treatment[a] (amount/100 gallons)	Twig lesions[b] (number)
Ziram 2 lb	6
Ziram 2 lb + Ortho Kleenup Flowable Emulsion 4 gal.	3
Ziram 3 lb	4
Ferbam 3 lb	7
Ferbam 3 lb + Ortho Kleenup Flowable Emulsion 4 gal.	1
Niacide Z 2 lb	3
Niacide Z 2 lb + Ortho Kleenup Flowable Emulsion 4 gal.	5
Dodine 2 lb	32
Orthocop 53 5 lb + Ortho Kleenup Flowable Emulsion 4 gal.	9
Bordeaux mixture 10-10	8
Unsprayed	66
LSD .05	33

[a] Sprays applied November 21, 1958 to seven randomized single-tree replicates.
[b] Lesions counted April 16, 1959 on 210 6-inch twigs per treatment.

Although the incidence of Coryneum blight during the winter of 1958-59 (Table 3) was very low, the efficacy of the dithiocarbamates in controlling this disease was again demonstrated. All of the materials tested significantly reduced the amount of disease, but the control obtained with dodine was mediocre. There was no evidence that a dormant oil emulsion, used in combination with the dithiocarbamates, affected the fungitoxicity of these materials in this test.

Peach fruit from plots sprayed with dithiocarbamates on November 25, 1959 was harvested July 21, 1960 and analyzed for the amount of active ingredient. The results of these analyses were as follows: ziram, 2 pounds/100 gallons, 0.13 ppm; ziram 2 pounds plus dormant oil emulsion 4 gallons/100 gallons, 0.12 ppm; ziram 3 pounds/100 gallons, 0.17 ppm; ferbam, 2 pounds/100 gallons, 0.25 ppm; ferbam 2 pounds plus dormant oil emulsion 4 gallons/ 100 gallons, 0.10 ppm.

All of the residues were well below the 7 ppm Food and Drug Administration tolerance for ziram and ferbam on peaches.

DISCUSSION

The research reported here indicates that ziram and ferbam can be used effectively as fall sprays for the control of Coryneum blight of peach under California conditions. In two of the test years precipitation during the critical period of late fall, winter, and early spring was approximately 70% of normal, but during the third year (1957-58) the rainfall during this period was more than double the normal. The present work corroborates the reports from Oregon (1, 2), Italy (4) and Argentina (7) on the effectiveness of these materials for controlling this disease. It also showed that adequate control could be obtained even though most of the ziram and ferbam had been lost from the trees during the 3 months immediately following spraying. Although a proprietary adhesive and oil emulsions increased somewhat the residual life of these materials, they failed to enhance disease control. Ziram and ferbam compared favorably with copper materials in controlling Coryneum blight, and ziram was significantly better than nabam (tank-mixed iron and zinc sulfates), captan, and dichlone. Since previous work (3) has shown that ziram and ferbam applied in the fall effectively control peach leaf curl, it now appears that these materials can be recommended for use in the fall to control both this disease and Coryneum blight. Residues of the active ingredients in the fruit were well within the Federal tolerance, and there was no evidence that these materials or any of the other fungicides included in these tests were phytotoxic to peach.

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MOSAIC, A NEW VIRUS DISEASE OF KALANCHÖE FLAMMEA

Harry C. Burnett and Ray A. Long[1]

Many species of Kalanchöe are grown outdoors in Florida and other tropical and subtropical regions of the world. In cool and cold areas they are popular indoor or greenhouse plants. They are commonly used in Florida in beds and borders in shady or semishady gardens. Because of their ease of cultivation, propagation, and rapid multiplication, these succulent plants are becoming more popular each year as new species and hybrids are introduced.

In November 1961 the writers, while inspecting a large tropical garden of miscellaneous plants at Lantana, Florida, noted a yellow-green mosaic pattern on the leaves of Kalanchöe flammea. Almost all the plants in two beds showed this unusual condition. That same day at West Palm Beach, Florida, more plants of K. flammea were found showing this same leaf pattern. Ten plants from Lantana were brought back to the Plant Pathology Laboratory at Winter Haven, to be used to conduct future studies.

The leaf symptoms were suggestive of those caused by virus infection in other plants; however, no virus disease in the genus Kalanchöe, or any in the family Crassulaceae, has been reported.

Tip cuttings from plants showing this condition were cleft-grafted on disease-free plants of K. flammea. In 24 days, identical symptoms were noted on the new growth of the inoculated plants.

SYMPTOMS

Symptoms may appear anywhere on new leaves as yellow-green streaks or blotches. Symptoms frequently appear first at or near the leaf margins and are often more numerous and pronounced than those found elsewhere on the leaf. Later, as the leaf matures, the yellow-green blotched areas enlarge and are scattered about the leaf. These areas may be elongated or, more commonly, irregular rounded, or circular areas or rings (Fig. 1). When infection is severe, a large part or almost the whole leaf may be involved. Usually no symptoms develop near the base of the leaf. On K. flammea there has been no leaf deformation nor any noticeable reduction in leaf size. Symptoms have not been observed on any other part of the plant except the leaves.

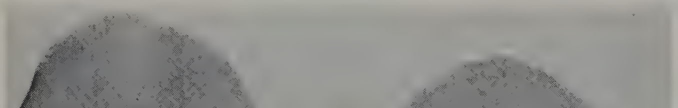

FIGURE 1. Mosaic symptoms on mature leaves of Kalanchöe flammea.

TRANSMISSION STUDIES

Three healthy plants of K. flammea were inoculated with infected grafts. The plants were cut back and two leaves just below the cut were retained. The conventional cleft-graft method was used and the infected scion was placed on the healthy understock. These were wrapped with 1/4-inch rubber strips commonly used in grafting. The plants were placed under a greenhouse bench beneath an intermittent mist, to take advantage of the high humidity. After 10 days the plants were placed on top of a greenhouse bench. First symptoms developed in 24 days. Symptoms have been observed as early as 15 days after inoculation, but usually at least 21 days are required and in some cases up to 40 days; this is dependent upon the initiation of new growth following inoculation.

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To date, K. flammea and six other species of Kalanchöe have been tested for susceptibility to this virus; K. aliciae, K. volutina, K. blossfeldiana, and K. schumacheri have been found susceptible. Symptoms expressed by all four species were identical. However, one additional symptom was observed on K. aliciae: severely infected leaves were deformed. No symptoms have been noted on K. scandens and K. marmorata during the 5 months they have been inoculated.

To date, all attempts to transmit Kalanchöe mosaic mechanically from diseased to healthy plants of K. flammea, K. aliciae, K. schumacheri, and K. volutina have failed. Freshly expressed juice from infected plants was rubbed on healthy plants that had been dusted with carborundum powder. No symptoms were noted during a 5-month period.

Host studies involving other species of Kalanchöe are now in progress.

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ADDITIONAL HOSTS OF THE FIG MOSAIC VIRUS

Harry C. Burnett[1]

In April 1958 several hundred fig plants, Ficus carica, from California and several hundred from Italy, grown in Florida under post-entry permit, were found to be infected with the fig mosaic virus. Studies were initiated to determine possible susceptibility to this virus disease of other moraceous plants growing in Florida.

Condit and Horne (2) first described the viral disease on Ficus carica and stated that it occurred on practically all varieties of fig wherever grown in California. They (3) listed Ficus altissima, F. krishna, and F. tsiela as hosts to this virus. Li and Procter (4) suspected F. retusa, F. repens, F. australis, and F. macrophylla of showing symptoms of the disease, but transmission from diseased to healthy plants was not demonstrated. Burnett (1) found Ficus lucescens (F. wightiana), F. jacquinifolia, F. rubiginosa (F. australis), F. retusa (F. nitida), F. mallatocarpa, F. glomerata, an unidentified species of Ficus, and Cudrania tricuspidata as hosts to this virus. C. tricuspidata is the first and only plant outside of the genus Ficus found to be susceptible to this disease. In this same paper, F. quercifolia and F. kirstingii were listed as showing no symptoms 6 to 15 months after inoculation. Later, after more plants were inoculated, symptoms did appear.

This paper reports six additional species of Ficus susceptible to the fig mosaic virus: F. quercifolia and F. kirstingii (PI 101320[2]) plus F. garciniaefolia (PI 260748), F. stricta (PI 268135), F. cocculifolia (PI 264684) and F. sp. (PI 269491). All plants showed mosaic leaf patterns similar to those as described on fig leaves by Condit and Horne (2), including at least some leaf distortion and reduction in leaf size. F. stricta showed extreme leaf distortion and greatly reduced leaf size. Some leaves were only one-tenth the size of normal leaves. F. garciniaefolia showed greatly distorted leaves, and some mosaic infected leaves were only two-thirds normal size. No fruit symptoms were noted on any of the above plants.

Acknowledgment: Appreciation is due Dr. Murray Gaskins, U. S. Plant Introduction Garden, Miami, Florida, for supplying many of the Ficus species used in these studies.

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THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 46 Number 10

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Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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ACCEPTANCE OF MANUSCRIPTS

The volume of pertinent material submitted for publication in the Plant Disease Reporter has made it necessary to limit the length of articles accepted to twelve double-spaced typed pages, including tables, graphs, and photographs. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases.

Paul R. Miller

SUGGESTIONS FOR PREPARATION OF MANUSCRIPTS

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

(2) MANUSCRIPTS should be the original ribbon copy, not carbons, clearly typed and double-spaced throughout, including tables, footnotes, and literature cited. Submission implies nonsubmission elsewhere and (if accepted) no publication elsewhere in the same form without consent.

(3) ABSTRACTS are requested for all except very short articles.

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CONTENTS

1. A foliar nematode disease of western sword-fern, Polystichum munitum
JAMES L. SANDENO and HAROLD J. JENSEN 699

2. The hop cyst nematode (Heterodera humuli Filipjev, 1934) found in Oregon
HAROLD J. JENSEN, et al. 702

3. An inexpensive heat chamber for curing virus diseased plants
PAUL R. FRIDLUND 703

4. Life cycle studies of North American rust fungi
JOHN W. BAXTER 706

5. Reaction of resistant and susceptible soybeans to Xanthomonas phaseoli var. sojensis
D. W. CHAMBERLAIN 707

6. Plant-parasitic nematodes associated with shortleaf pine showing symptoms of littleleaf
JOHN L. RUEHLE 710

7. Pathogenicity of Pratylenchus vulnus on boxwood, Buxus sempervirens var. arborescens
W. W. OSBORNE and W. R. JENKINS 712

8. Recorded Dutch elm disease distribution in North America as of 1961
FRANCIS W. HOLMES 715

9. Postharvest deterioration of the downy mildew-susceptible lettuce variety Great Lakes and the resistant variety Calmar
F. W. ZINK and J. E. WELCH 719

10. Rose as a source of Prunus ringspot virus
H. C. KIRKPATRICK, et al. 722

11. The effect of powdery mildew on the growth of Kentucky bluegrass
T. A. GASKIN and M. P. BRITTON 724

12. Particularly significant specimens of Elsinoë and Sphaceloma taken by U. S. Plant Quarantine Inspectors
ALICE J. WATSON and ANNA E. JENKINS 726

13. A severe outbreak of Selenophoma spot on 'Gaines' wheat
C. S. HOLTON and LAURENCE H. PURDY 728

14. Stagonospora bromi on Bromus inermis
K. E. ZEIDERS and J. H. GRAHAM 729

15. Tupelo lesion caused by Fusarium solani
E. RICHARD TOOLE 732

16. Fomes root rot found in five thinned, native white pine stands in West Virginia
JAMES H. GINNS and WILLIAM H. GILLESPIE 734

17. Movement of the burrowing nematode from a potted Duncan grapefruit seedling into previously uninfected seedlings growing in adjacent pots
E. B. SLEDGE 735

18. The natural occurrence of Fomes annosus and associated fungi in slash pine stumps
J. S. ASTIN, Jr. and CHAS. H. DRIVER 738

19. A method for assaying nematocidal activity of experimental chemicals
D. E. JOHNSON and BERT LEAR 742

20. Stemphylium leaf spot of cotton reported from Louisiana
J. B. SINCLAIR and M. N. SHATLA 744

21. Effects of paper and sawdust amendments on meadow nematodes and subsequent Verticillium wilt of tomatoes
P. M. MILLER and L. V. EDGINGTON 745

22. Fusarium wilt of safflower
JOHN M. KLISIEWICZ and BYRON R. HOUSTON 748

23. Mexican palm trees apparently killed by Penicillium vermoeseni
T. R. CARPENTER, et al. 750

24. Tuberculina maxima on white pine in Michigan
WILLIAM J. YOUNG 750

25. Corrections 750

A FOLIAR NEMATODE DISEASE OF WESTERN SWORD-FERN, POLYSTICHUM MUNITUM[1]

James L. Sandeno and Harold J. Jensen

Abstract

The decorative beauty of the western sword-fern, Polystichum munitum, a favorite cut fern of the florist trade, can be reduced or destroyed by foliar nematodes. Infection by the pest identified as Aphelenchoides fragariae (Ritzema Bos, 1891) Christie, 1932 results in water-soaked wedge- or band-shaped areas on the pinnae which darken as the season progresses. Although several investigators have observed this pest on various ferns, including the western sword-fern, a discussion of its discovery, distribution, and symptomatology has not been published.

Fern fronds are important items in the florist "greenery" trade which derives an estimated $5,000,000.00 yearly income from Pacific Northwest forested areas. The fern most commonly used is the western sword-fern, Polystichum munitum. The fronds are picked from their natural forest habitats in the Cascade and Coast ranges, then taken to local packing plants where they are processed and packed as a standard florist commodity. Several types of discoloration may develop in refrigerated storage or in transit which lessen the decorative beauty of the frond and result in economic losses to suppliers. One type of discoloration is caused by foliar nematodes belonging to the genus Aphelenchoides.

Most of the literature pertaining to Aphelenchoides spp. found in fern lists the nematode as Aphelenchoides olesistus (Ritzema Bos, 1893) Steiner, 1932,(6, 7, 9, 11, 12, 15, 16). Many investigators now consider Aphelenchoides olesistus to be a synonym of Aphelenchoides fragariae (Ritzema Bos, 1891) Christie, 1932 (1, 3, 5, 13, 14). In agreement with this synonymy and according to Allen's classification (1) we have identified the nematode in western sword-fern as Aphelenchoides fragariae.

Shaw's host index for the Pacific Northwest (12) and the host index of plant diseases compiled by the United States Department of Agriculture (15) list Polystichum munitum as a host for Aphelenchoides olesistus, but no actual report of occurrence or description of symptoms could be found. Several investigators had observed various leaf discolorations on western sword-fern before nematodes were discovered in fern pinnae. Consumer complaints over undesirable leaf discolorations caused the greenery industry to seek advice and assistance from the United States Department of Agriculture and local universities. H. P. Barss, Drs. F. P. McWhorter (Oregon Agricultural Experiment Station) and Freeman Weiss (United States Department of Agriculture) investigated the problem in 1931-33, but failed to establish the cause. It was not until 1944, however, that the late Dr. G. Steiner discovered that leaf nematodes were often associated with leaf discoloration. A recent personal communication from Edna M. Buhrer (Nematologist, Nematology Investigations, Beltsville, Maryland) indicates that Dr. Steiner's observations and identification were not formally published but were noted in their nematology records as of June 1944. Miss Buhrer also calls our attention to the fact that Drs. Steiner, Emsweller, and McWhorter obtained their material from the California State Redwoods Forest near Crescent City, California, and from a private estate in San Francisco, California. The original collection of Christmas or western sword-fern known to have contained nematodes, however, was received by H. P. Barss from Chehalis, Washington in December 1931.

This paper is presented to: (a) preserve the historical background of finding the disease, (b) verify that the western sword-fern, Polystichum munitum, is a host of Aphelenchoides fragariae, (c) verify occurrence of the disease in Oregon, and (d) describe symptoms of the disease.

MATERIAL AND METHODS

Observations were made of material personally collected from the fern's natural forest habitat and of material supplied by a packer of greenery. Although fern fronds were obtained from several areas from July 1960 to March 1962, most were collected from a forest area approximately 8 miles east of Triangle Lake, Oregon (just off State Highway 36) and from Mary's Peak, Oregon. Additional fern fronds were supplied by Callison's, Inc. from their packing plant in Eugene, Oregon. Most of these fronds were picked in the coastal mountains of western Oregon.

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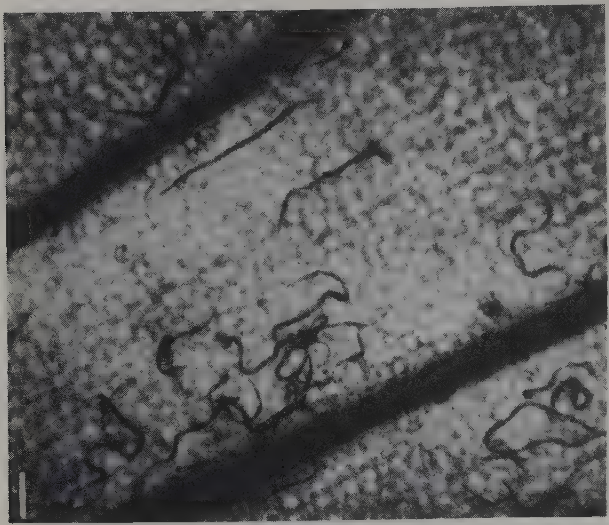
[1] From a portion of a study supported by Callison's, Inc., Seattle, Washington.



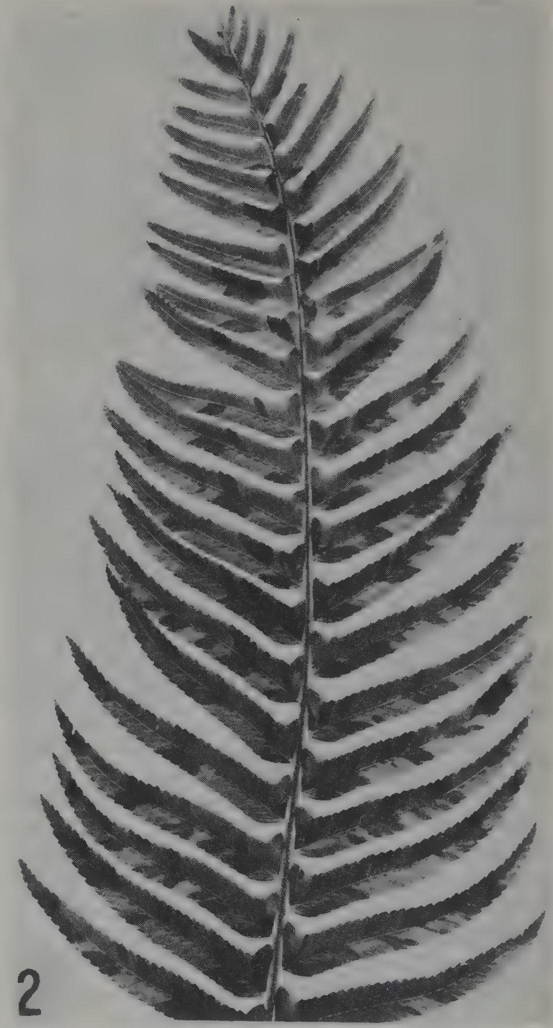


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FIGURE 1. Pinnae section of western sword-fern, Polystichum munitum, stained and magnified to show foliar nematodes, Aphelenchoides fragariae (Ritzema Bos, 1891) Christie, 1932, in host tissue.

FIGURE 2. Foliar nematode symptoms of band- and wedge-shaped blotches on pinnae of a western sword-fern frond. (Photograph courtesy of Millsap and McWhorter, Oregon State University, and Nematology Investigations, U. S. Dept. of Agr.)

Nematodes were isolated from fern tissue in three ways: (a) by shredding tissue in water with dissecting needles, (b) by placing pinnae in water for 18 hours to allow the nematodes to migrate from tissue into the surrounding water, and (c) by separating the nematodes from tissue processed in a Waring Blendor and extracted with a Baermann funnel. Samples of the nematode population were placed in temporary slide mounts and identified.

The method used for staining nematodes within the pinnae tissue was similar to that described by Goodey (8) and Franklin (4). Nematodes were readily isolated from water-soaked areas and the lactophenol-acid fuchsin technique for staining these tissues revealed the presence of numerous nematodes within the pinnae (Fig. 1). Isolation techniques and staining showed that nematodes are rarely present in brown discolored tissues.

SYMPTOMS AND SEASON DEVELOPMENT

Characteristic symptoms of nematode infection consist of band- to wedge-shaped blotches on the pinnae (Fig. 2). Initially the blotches appear as water-soaked areas on the undersurfaces of the pinnae, but as symptoms progress these sites become a dull brownish color or may disappear. Finally the original infection sites appear as dark-brown wedge-shaped bands occurring on both the upper and lower pinnae surfaces. The typical shape of the blotches appears to be influenced by the lateral veination of the pinnae. Apparently nematodes favor interveinal spaces as the mid- and lateral veins appear to impede the spread of infection. Much of the ultimate dark coloration may be due to a fluorescent Pseudomonas bacterium found in association with the nematodes.

Symptoms appear on the pinnae of western sword-fern during late January. At this time, and throughout February and March when the forests are very damp, symptoms occur as water-soaked bands on the undersides of old growth pinnae (last season's fronds). As the forests dry in May and new fronds begin to appear as fiddlenecks, the original water-soaked blotches become brownish in color. The dark brown, wedge-shaped bands replace the water-soaked areas in June as the forests become dry. Symptoms were observed on both pinnae surfaces by this time. Although the new fronds had uncurled by mid-June, no water-soaked or brownish areas were seen until the following winter.

Nematodes found in the fern tissues in January and February were larvae. Adults were not seen until March when all stages could be found.

DISCUSSION

The symptoms of foliar nematodes in western sword-fern are most noticeable during the winter months when vegetative growth is slow. During the spring and summer months, when fern growth is most active, the symptoms are masked. This relationship between fern growth and visible symptoms caused by nematodes follows the same sequence that has occurred with other Aphelenchoides spp. on ferns grown in ferneries (7). The early water-soaked symptoms that occur on western sword-fern also seem to be associated with excess moisture during winter rains.

Most references in the literature dealing with Aphelenchoides spp. on fern do not mention a water-soaked symptom, but state that the infected tissues are characterized by blotched or sharply defined dark brown or black discoloration (7, 9, 10, 11, 16). Ark and Tompkins, however, described a nematode disease of bird's-nest fern in which the first symptoms of nematode infection consisted of a slight water-soaked condition and that this symptom soon disappeared. The tissue then turned a dull brownish-black, and the nematodes and accompanying discoloration spread rapidly, often killing affected plants (2). The pattern of nematode injury to western sword-fern follows that of bird's-nest fern except that the discoloration does not spread and the plants are not killed.

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THE HOP CYST NEMATODE (HETERODERA HUMULI FILIPJEV, 1934) FOUND IN OREGON

Harold J. Jensen, Harriet R. Smithson, and Lindsey B. Loring[1]

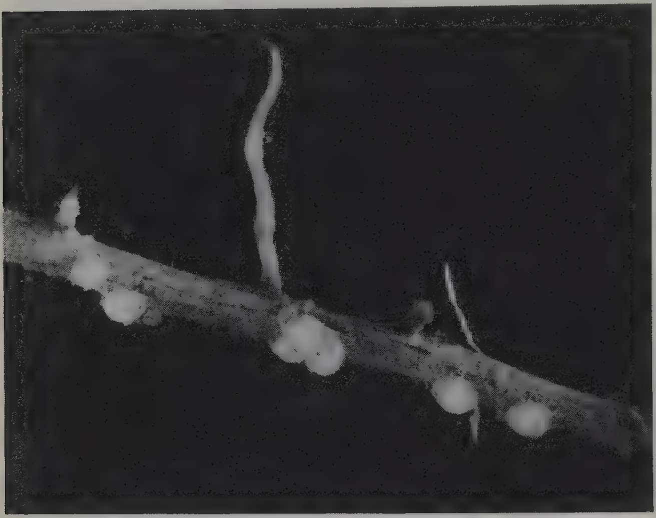

FIGURE 1. White cysts of the hop cyst nematode (Heterodera humuli Filipjev, 1934) on a portion of Backa hop root (Humulus lupulus).

The recent interception of the hop cyst nematode[2] at Seattle, Washington by United States Department of Agriculture Plant Quarantine Inspectors W. Brown and S. Nakahara initiated a chain of events that eventually led to discovery of the nematode in Oregon hop yards. Initial surveys proved the pest occurs in yards near Independence and Salem, Oregon. Soil samples from these loci contained numerous larvae and empty-, partly filled- or full cysts. White cysts (immature females) were found on hop roots (Fig. 1) of Backa, Brewer's Gold, Bullion, Fuggles, and Kent varieties. Dimensions of females, cysts, and larvae fall well within the range stated in the species' description. Since distribution and variety host surveys were only preliminary, other locations and varieties are probably invaded. The exchange and movement of hop roots from yard to yard and area to area, combined with annual flooding, have probably caused a wide distribution of the pest. The fact that hop roots have been an international exchange item for many years makes determination of the original source of infection extremely difficult.

Information about this pest is very limited and is almost entirely dependent upon European sources. Heterodera humuli was found first in Germany, later in England, and more recently in Belgium, Italy, Union of South Africa, and United States (State of Washington) (1, 2, 3, 4). The reported host range, in addition to hops (Humulus lupulus), is hemp (Cannabis sativa) and nettle (both Urtica dioica and U. urens). The importance of this pest to hop culture is not known, but European nematologists believe the nematode has very little effect upon yield. The appearance of white cysts on the roots is the only abnormal condition we observed on infected hop plants. Past records indicate that the Oregon yards, now known to be infested, have yielded normally. Additional surveys, pathogenicity and host-range studies are in progress.

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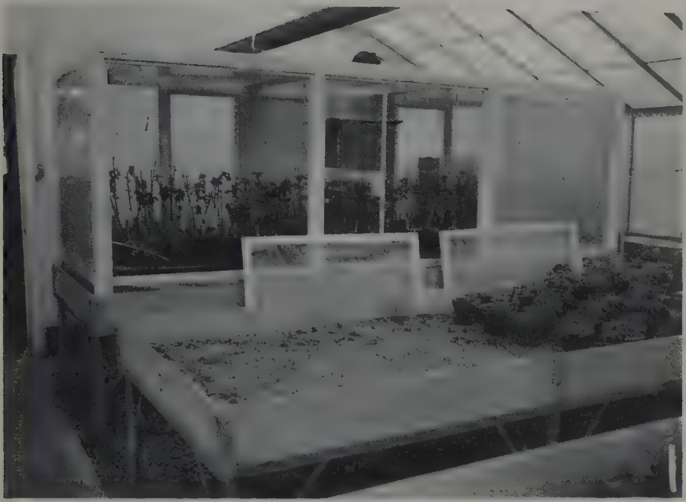
[2] Confirmed by G. S. Cobb, Nematology Investigations, Plant Industry Station, Beltsville, Maryland.

AN INEXPENSIVE HEAT CHAMBER FOR CURING VIRUS DISEASED PLANTS[1]

Paul R. Fridlund[2]



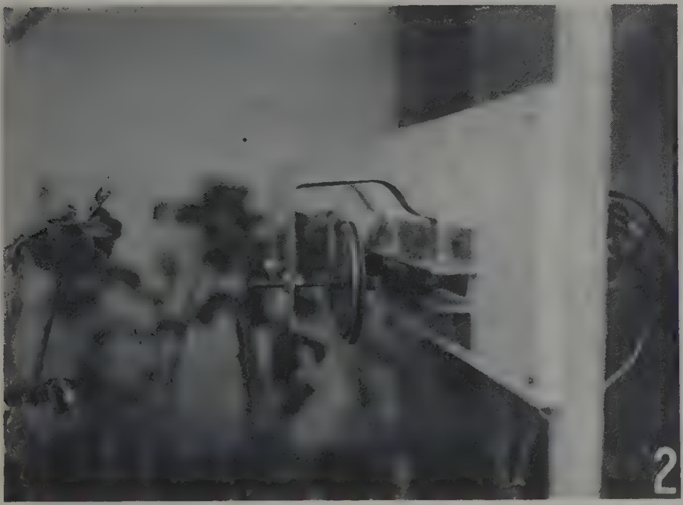


FIGURE 1. Heat chamber located on a 4 x 12 foot bench with detachable service panels removed.

FIGURE 2. The heating and venting controls consist of a series of small snap action switches and ether wafers used principally in chicken brooders.

Thermal inactivation of virus in plants has been used successfully against a number of viruses in various species. In Prunus, two methods of heat application have been employed: 1) immersing diseased budwood in a constant temperature hot water bath for a number of minutes (1, 2, 5, 8, 10, 12), and 2) exposure of diseased plants to hot air at about 100° F for much longer intervals, usually 2 to 8 weeks (4, 5, 9, 11). Apparently the hot water treatment is effective against some viruses, but not all, as it has been shown ineffectual in some instances (3, 6, 7, 8). On the other hand a wider range of effectiveness has been obtained with hot air treatment, since it inactivates some of the viruses not inactivated by hot water. Unlike the equipment used in hot water treatment, that used in hot air treatment is more complex, and thus can be expensive. This paper describes an effectual heat chamber for hot air treatment which 1) is large enough to contain small trees, 2) can be built cheaply, and 3) has been used successfully with virus-infected Prunus.

There are three environmental considerations in growing plants in a heat chamber: light, humidity, and temperature. The controls and equipment for each are usually expensive. In the described equipment, a light source can be ignored because the chamber is constructed of a translucent material and is located in a greenhouse. Apparently precise humidity control is not necessary for successful heat treatment. Therefore, control within a reasonable range is usually possible through normal watering, heating and ventilating practices. Air temperature appears very critical in some virus inactivations, and control within about 1° ± F is desirable. Apparently there is a very narrow range between temperatures at which some viruses are inactivated and host tissues are killed.

The chamber has gross dimensions of 4 x 4.5 x 12 feet and is simply constructed using 2 x 2 inch dimensional lumber (Fig. 1). It is covered inside and out with 4 mil polyethylene sheeting. Care was taken to cover completely the internal wood framing with polyethylene to

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[1] Washington Agricultural Experiment Station, Pullman, Washington. This work was conducted under Project 1262 and was supported by Interregional Project IR-2.

[2] Associate Plant Pathologist and Plant Pathologist in charge, IR-2, Department of Plant Pathology, Washington State University, Irrigation Experiment Station, Prosser, Washington.

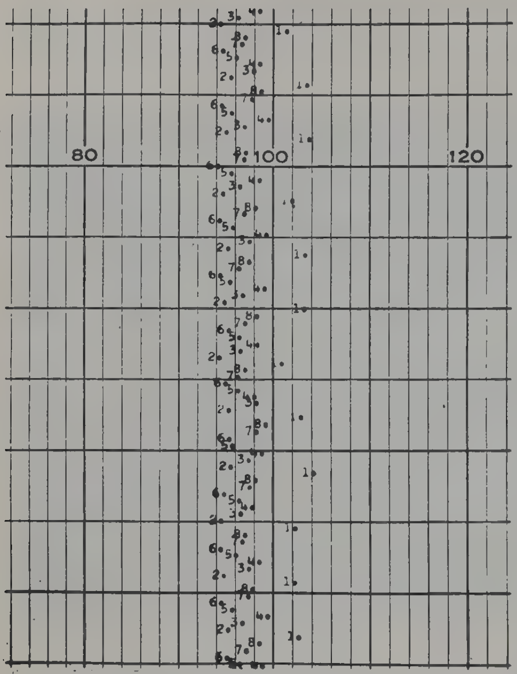

FIGURE 3. Normal operating temperatures within the heat chamber. Thermocouple # 1 was in open air, and the other seven were placed beneath peach buds chip budded to potted seedling peach rootstocks.

prevent water-soaking. The chamber shown was made in five panels (four sides and the top) held together with lag screws for easy disassembling. The figure shows the detachable portions of one panel used for convenient service access. The opposite side contains the electrical controls and two small doors for access for watering the plants during the treatment period.

Heat is provided by four 660 W at 120 V heating cones located inside near the top of a 7 inch x 14 inch x 4 foot transite duct. The duct can be seen on the opposite, right center side of the chamber in Figure 1. A 45 or 60 CFM utility blower running continuously at the base of the duct forces cool air up and over the heating cones, whether they are heating or not, for proper air circulation. A 1-inch opening around three sides of the top of the duct allows hot air to be forced out and distributed evenly; otherwise the top of the duct is covered. The whole duct is lined with a heavy gauge aluminum foil for more even heat distribution. A solid shelf on which to set the potted plants is spaced within the chamber about 8 inches above the greenhouse bench. Since the air circulating utility blower is located below the shelf, a cold air return is provided by leaving a 1-inch space around the periphery of the shelf.

Cooling is provided through the top of the chamber, with the whole top serving as a polyethylene duct. Cool air is forced into the top with another utility blower (140 CFM) and is discharged inside the chamber through a series of rows of 1-inch holes in four plywood strips. The strips nearest the ends have more holes to lessen air resistance and thereby provide more even distribution of cool air.

The temperature control is a simple and economical arrangement consisting of one expansion-type ether wafer designed for chicken brooder use, and an Acro, snap action, single pole-double throw, 15 A at 125 V, switch (Fig. 2). The long bolt upon which the wafer is mounted is used to adjust the temperature setting. At the temperature used, 100°F, this type of control is very accurate.

The operating procedure is as follows: Two heat cones (set no. 1) are wired through one side of the double throw switch, and the cooling vent fan (140 CFM) is wired through the other. Thus, the chamber is continuously heating or venting, depending upon the relationship of the actual temperature to the set temperature. Since only two cones may be run off of one 15 A switch, an identical set of controls is used to control the other two heating cones (set no. 2), except a vent fan is not connected.

The most effectual temperature setting for the latter cones (set no. 2) will vary with the environment in which the heat chamber is located. In our experience it was found that a setting of about 1° F lower than the other cones (set no. 1) prevented excessive heating but also resulted in an accumulation of free water on the chamber walls through reduced venting. Therefore the setting of cone set no. 2 was raised to about 102° F, and the control's only function was to prevent accidental overheating. Meanwhile venting capacity was increased to 140 CFM to prevent any overheating since in actual practice the vent fan was always operating against two heat cones. The increased venting thus obtained controlled the excessive humidity.

The chamber has been maintained in a 78° F external environment. Experimentation has shown that the chamber does not lose temperature control until external temperatures reach 72° or below. At these temperatures the heating cones do not have enough capacity to maintain the chamber at 100°. Additional heat cones would permit chamber operation at lower external temperatures.

To determine the exact temperature fluctuation within the chamber, and the internal temperatures of trees exposed to these temperatures, an eight-thermocouple Leeds and Northrup, Speedomax Type G, Model S, 60,000 series recorder was used. Seven calibrated thermocouples were placed beneath chip buds of peach which had been grafted to potted peach rootstocks, and one was left in the open air. Thermocouple #1 was the one in open air. The results are shown in Figure 3. The recordings show that the average set temperature during the test period was about 102° F. The total air temperature variation approximated 2°. A scalloped effect in the recorded pattern was correlated with the alternate heating and venting of the chamber. The thermocouples placed under buds indicated that the average plant temperature was about 6° less than the surrounding air temperature, and that there was a variation between plants of about 5°. Why inter-plant variation occurred is not known. It would appear plausible that those plants that have the lowest average temperatures are those that failed to be heat cured during a specific heat treatment period.

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LIFE CYCLE STUDIES OF NORTH AMERICAN RUST FUNGI[1]

John W. Baxter

This paper reports the results of recent research on the life cycles and alternate host relationships of three rust fungi occurring in the Great Lakes region, the upper Midwest and the Plains States. This study included field observations, field inoculations at Milwaukee, Wisconsin, and controlled inoculations in the greenhouse at the University of Wisconsin -- Milwaukee, using rust material collected in the field and host plants grown in the greenhouse from rust-free seed or rhizomes. Uredial and aecial specimens resulting from successful inoculations have been deposited in the Arthur Herbarium, Purdue University.

PUCCINIA SPOROBOLI Arth. var. ROBUSTA Cumm. & Greene

The occurrence of this rust on Calamovilfa longifolia was first reported by Cummins and Greene (3). Previously, all rust collections on C. longifolia had been referred to Puccinia amphigena Diet. In the sand dune area along the Lake Michigan shore south of Sheboygan, Wisconsin, rust infection on C. longifolia is usually abundant, consisting of a mixture of Puccinia sporoboli var. robusta and P. amphigena. In May 1960, overwintered telial material on C. longifolia from this area was used to inoculate young rust-free plants of Yucca glauca, Yucca elata, Smilacina stellata, and Sansevieria sp., resulting in the production of spermagonia and aecia on the two species of Yucca and Smilacina stellata, and spermagonia only on Sansevieria sp. The aeciospores from Yucca glauca and Smilacina stellata were then used in successful inoculations of plants of Calamovilfa longifolia grown in the greenhouse from rust-free rhizomes. Microscopic examination of urediospores from the resulting uredia showed them to be typical of P. sporoboli var. robusta.

In later studies, overwintered telial material of P. sporoboli var. robusta, collected at the type locality of this variety, near Burns, Wyoming, was used to inoculate seedlings of Yucca baccata, Yucca filamentosa, and Yucca brevifolia (Joshua tree). Aecia were produced on all three species of Yucca.

PUCCINIA PHRAGMITIS (Schum.) Koern.

Known aecial hosts of this rust include Rheum rhaponticum (rhubarb) and species of Rumex. In 1958 Baxter (2) reported obtaining weak infection on Fagopyrum esculentum (buckwheat), using telial material from Nebraska. In May 1961, overwintered telial material collected near Missouri Valley, Iowa was used to inoculate young plants of buckwheat, resulting in abundant development of spermagonia and aecia of normal size.

PUCCINIA ANDROPOGONIS Schw.

Successful inoculation of a seedling of Andropogon sp. with aeciospores of Aecidium aesculi Ell. & Kell. was reported by Baxter (1) in 1955. Recently the reciprocal inoculation was made, using telial material of Puccinia andropogonis collected near Urbana, Illinois. Inoculation of young leaves of Aesculus glabra (Ohio buckeye) was followed by abundant production of spermagonia and aecia on this host.

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REACTION OF RESISTANT AND SUSCEPTIBLE SOYBEANS TO XANTHOMONAS PHASEOLI VAR. SOJENSIS[1]

D. W. Chamberlain[2]

Abstract

Dilutions between 1-1,000 and 1-10,000 of a standard suspension (about 800,000,000 cells/ml) of Xanthomonas phaseoli var. sojensis were satisfactory for evaluating bacterial pustule reaction of greenhouse plants. The organism multiplied in the leaves of the resistant CNS variety but at a lower rate than in those of the susceptible Lincoln. In leaf extracts, there was no consistent difference in rates of multiplication of the organism in extracts from resistant and susceptible leaves. Grafting resistant scions to susceptible roots and vice versa did not affect the susceptibility of the leaves. Bacteria-free culture extract did not stimulate pustule formation in susceptible soybean leaves. Passage of the organism through the leaves of the resistant variety had no apparent effect on its pathogenicity.

INTRODUCTION

In 1943, Hartwig and Lehman (1) found that the soybean variety CNS showed no evidence of bacterial pustule, in spite of a generally heavy natural infection on other soybean varieties. Artificial inoculations during the next two seasons with the causal organism, Xanthomonas phaseoli var. sojensis (Hedges) Starr & Burkh., indicated that CNS was virtually immune from bacterial pustule. Since this variety is the best source of resistance to bacterial pustule, it has been used widely as a parent in breeding for disease resistance. This paper reports the results of experiments on host-parasite relations and on the nature of resistance to X. phaseoli var. sojensis in CNS.

MATERIALS AND METHODS

Inoculations were made by forcibly spraying inoculum against the underside of the leaf with an atomizer attached to a compressed-air line until water-soaking of the leaf tissue was visible. To aid in making counts, each leaflet was inoculated at six points to provide 18 individual inoculations per trifoliolate leaf. Plants in the 3- or 4-trifoliolate leaf stage were inoculated on the uppermost fully developed leaf. Inoculum was made from X. phaseoli var. sojensis grown 24 to 48 hours on Wernham's buffered PDA (potatoes, 200 g; dextrose, 20 g; agar, 18 g; KCl, 0.20 g; $NaNH_4HPO_4$, 0.50 g; NaH_2PO_4, 0.17 g; distilled water, 1 L.). The bacteria were suspended in sterile distilled water and adjusted to conceal a 2-mm loop of No. 26 B. & S. gauge wire at a depth of 12 mm. Thus standardized, the suspension had about 800,000,000 cells/ml. Greenhouse temperatures varied from 24° to 30° C during the experiments.

EXPERIMENTAL RESULTS

Effect of Inoculum Dilution on Host Reaction: In field tests for evaluating the reaction of soybean strains to X. phaseoli var. sojensis, an inoculum concentration of 8,000,000 or more cells/ml is commonly used. This inoculum, however, usually causes excessive necrosis on greenhouse plants. Accordingly, tests were made on the resistant CNS and the susceptible Lincoln variety with various dilutions of inoculum to determine the effect of bacterial concentrations on the development of pustules, necrosis, and chlorosis.

The standardized suspension of X. phaseoli var. sojensis was diluted as follows: 1-100, 1-1,000, 1-10,000, 1-100,000, 1-1,000,000, and 1-10,000,000. Three plants of Lincoln and of CNS were inoculated with each dilution of inoculum. Inoculated spots showing pustules, necrosis, and chlorosis were counted 10 to 12 days after inoculation. The test was repeated twice to check the original results.

The averaged results of the three trials are shown in Table 1. Necrosis was obviously of no value in distinguishing resistant and susceptible reactions, since CNS showed more necrosis

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[1] Publication No. 381 of the U. S. Regional Soybean Laboratory, Urbana, Illinois.

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Table 1. Reaction of soybean varieties Lincoln and CNS to Xanthomonas phaseoli var. sojensis at different dilutions of inoculum.

Variety and inoculum dilution	% spots with pustules	% spots with necrosis	Necrotic spot size	% spots with chlorosis
Lincoln:				
1-100	100	100	large	100
1-1,000	83	77	medium	91
1-10,000	61	50	small	63
1-100,000	5	55	small	52
1-1,000,000	15	30	small	33
1-10,000,000	0	24	small	40
CNS:				
1-100	0	100	large	100
1-1,000	0	66	large	22
1-10,000	0	98	small	60
1-100,000	0	83	small	13
1-1,000,000	0	75	small	13
1-10,000,000	0	20	small	4

Table 2. Multiplication of X. phaseoli var. sojensis in leaves of resistant and susceptible soybeans.

Variety	No. viable bacteria per 4-mm leaf disk per indicated period after inoculation: 1 day	3 days	15 days	25 days
Lincoln	12,500	43,200	1,311,700	1,967,000
CNS	5,750	6,700	240,800	1,367,000

at the higher dilutions than did Lincoln. Chlorosis appeared to be associated with the susceptible reaction. When inoculum dilutions 1-1,000 to 1-10,000 were used, pustules formed abundantly on the susceptible Lincoln; moreover, the necrotic spots were smaller and did not obscure the pustules. Since the development of pustules is obviously the criterion of susceptibility, an inoculum dilution greater than 1-10,000 is unreliable, as indicated by the abrupt decrease in pustule formation between dilutions of 1-10,000 and 1-100,000.

Multiplication of X. phaseoli var. sojensis in Leaves of Resistant and Susceptible Soybean Varieties: Tests were made to determine whether the organism could multiply within the leaves of resistant CNS. The uppermost fully developed leaves on three CNS plants were water-soaked with a 1-1,000 dilution of the standardized inoculum. Three susceptible Lincoln plants were similarly inoculated. After water-soaking had disappeared, the inoculated leaves were rinsed with distilled water to remove bacteria adhering to the leaf surface. Bacterial populations were determined 1, 3, and 15 days after inoculation by cutting disks from the inoculated leaves with a 4-mm cork-borer. Six disks, two from an inoculated leaf from each plant, were macerated in sterile distilled water and allowed to stand for 20 minutes with frequent manual shaking. Serial dilutions were plated on Wernham's buffered PDA with 6 to 8 plates for each dilution. This test was repeated twice.

The average bacterial counts for three tests are shown in Table 2. The organism multiplied in the leaves of resistant CNS but at a lower rate than in those of susceptible Lincoln. Moreover, high populations of viable bacteria were still present in the resistant leaves 25 days after inoculation.

Multiplication of the Organism in Leaf Extracts from Resistant and Susceptible Plants: Resistant plants were tested for the presence of an inhibitor. Leaves of CNS were ground in a food-grinder and the expressed juice was centrifuged and Seitz-filtered. A leaf extract from the susceptible Lincoln was similarly prepared. The extracts were placed in tubes, 5 ml per tube and four tubes for each extract, and inoculated with a 24-hour culture of X. phaseoli var. sojensis. The extracts were also tested in dilutions of 1-1 and 1-3 parts water. Platings were made on PDA 1, 3, 6, and 15 days after inoculation to determine bacterial multiplication rates.

The test was repeated four times.

The bacteria multiplied in both resistant- and susceptible-leaf extracts; maximum multiplication occurred between 3 and 6 days after inoculation. No consistent difference in growth rate of bacteria was evident in extracts from resistant and susceptible leaves. Although the population was greatly reduced, viable bacteria were plated from both extracts 2 months after inoculation.

Effect of Grafts between Susceptible and Resistant Plants on Susceptiblity to *X. phaseoli* var. *sojensis*: The possibility that materials affecting resistance might be translocated from the roots was explored. Susceptible (Lincoln) scions were grafted to resistant (CNS) rootstocks and resistant scions were grafted to susceptible rootstocks before the primary leaves were fully developed. Three weeks later, six of the best plants from each rootstock-scion combination were inoculated on the uppermost fully developed leaves with *X. phaseoli* var. *sojensis*. The plants were examined 7, 12, and 18 days later.

Pustules developed on the Lincoln scions growing on the CNS rootstocks, but no pustules developed on the CNS scions growing on the Lincoln rootstocks. The experiment was repeated twice with the same results. Evidently resistance or susceptibility is a quality inherent in the leaves and is not modified by materials translocated from the roots.

Test for the Production of Growth Substance by *X. phaseoli* var. *sojensis*: Because of the characteristic hypertrophy and hyperplasia (2) induced by *X. phaseoli* var. *sojensis* in the mesophyll cells of susceptible soybean leaves, it seemed possible that the organism might produce a growth substance. Accordingly, the organism was grown in nutrient broth for 6 days and then centrifuged. The liquid was Seitz-filtered to eliminate bacterial cells. The pelleted bacteria in the centrifuge tube were washed through three changes of distilled water and suspended in distilled water.

The Seitz-filtered broth was diluted 1-1 and 1-10 with distilled water. Six Lincoln plants were inoculated with each dilution of the Seitz-filtered broth, and a similar number were inoculated with 1-1 and 1-10 dilutions of sterile broth in which no organisms had grown. A third series of Lincoln plants was inoculated with the washed bacteria. The plants were examined 3, 7, and 14 days later.

Chlorosis and necrosis were induced by the 1-1 dilution of the filtered broth, indicating some toxic effect. The sterile broth in which no organisms had grown induced no necrosis or chlorosis. No pustules developed from any of the broth inoculations. Pustules developed only on plants inoculated with the washed bacteria. The experiment was repeated with similar results. Evidently no growth substance capable of inducing hypertrophy or hyperplasia in the mesophyll cells is formed by the organism in nutrient broth. Millar (3), however, found that the organism produced an auxin when grown in a medium with L-tryptophan as the sole source of nitrogen.

Effect of Passage through Leaves of a Resistant Variety on the Pathogenicity of *X. phaseoli* var. *sojensis*: The effect of the resistant host on the pathogenicity of *X. phaseoli* var. *sojensis* was determined. Leaves of CNS on intact plants were inoculated and incubated for 10 days. Leaves of the susceptible Lincoln variety were similarly treated as a control. The organism was then recovered by crushing infected portions of the leaves in distilled water and straining out the coarse material with absorbent cotton. The strained suspension was then introduced into a second series of resistant and susceptible soybean leaves. After 10 days' incubation, the process was repeated with a third series of resistant and susceptible plants. The pathogenicity of *X. phaseoli* var. *sojensis* was tested after the second and third passages by isolating the bacteria from CNS and from Lincoln, respectively, on agar plates and inoculating Lincoln and CNS with standardized suspensions of each isolate.

Although three separate isolates of *X. phaseoli* var. *sojensis* were tested, no difference in pathogenicity was detected as a result of passage through the resistant variety.

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PLANT-PARASITIC NEMATODES ASSOCIATED WITH SHORTLEAF PINE SHOWING SYMPTOMS OF LITTLELEAF

John L. Ruehle[1]

In portions of the South and Southeast, littleleaf of shortleaf pine (Pinus echinata) is the most serious disease of this economically important species (5). The nature of the disease symptoms suggests some type of root injury leading to a nitrogen deficiency, or deficiency of some other essential element.

While early investigators were attempting to establish a cause for littleleaf, several workers investigated the nematodes associated with the extensive root injury connected with the disease. In March 1948 Harold Reynolds[2] collected root and soil samples from littleleaf-diseased areas in the vicinity of Athens, Georgia. He found the following plant-parasitic genera in both diseased and healthy shortleaf stands: Criconema, Hemicycliophora, and Hoplolaimus. In the same year, Jackson (2) reported that Criconemoides rusticum (Micoletzky) Taylor was associated with the deterioration of fine roots and mycorrhizae of shortleaf pine in stands with typical littleleaf.

Subsequently, Campbell and Copeland (1) presented evidence that littleleaf symptoms develop from the failure of the trees to absorb sufficient nitrogen because of a heavy mortality of feeding roots caused by Phytophthora cinnamomi Rands. Zak (6) showed that root injury on trees growing in heavy and poorly drained soils resulted not only from fungus attack, but from effects of poor soil aeration. He also concluded that damage from P. cinnamomi was accentuated when soil conditions were unfavorable for root regeneration.

Root diseases of this type usually involve complex interactions of many factors (3). Plant-parasitic nematodes might be responsible for the tree's inability to replace lost roots and thus contribute to the etiology of the littleleaf disease.

The two main objectives of the study reported here were as follows: 1) to determine what plant-parasitic nematode species are associated with roots of shortleaf pine exhibiting symptoms of littleleaf disease, and 2) to establish any correlation between a given nematode species and littleleaf by sampling within diseased and healthy stands.

Twelve counties throughout the Piedmont, from Virginia to Alabama, known to have shortleaf pine stands on severe littleleaf sites were selected at random. One diseased and one healthy stand (both at least 5 acres in size and of approximately the same age and soil type) were sampled in each selected county. Five separate soil samples were collected within each stand. Each sample was thoroughly mixed and teaspoon aliquots were processed according to a modified Baermann technique (4).

The five species of plant-parasitic nematodes commonly associated with littleleaf-diseased shortleaf pine were: the American dagger, Xiphinema americanum; sheath, Hemicycliophora vidua; spiral, Helicotylenchus dihystera and H. erythrinae; and lance, Hoplolaimus galeatus. Mean population values for both littleleaf and healthy stands are presented in Table 1. Other genera and species of plant-parasitic nematodes, such as Criconemoides sp., Longidorus sp., Meloidodera sp., Meloidogyne sp., Paratylenchus sp., Pratylenchus zeae, Trichodorus christiei, Trophurus sp., Tylenchorhynchus claytoni, and Xiphinema chambersi, were either recovered from a few samples in high numbers or were found frequently only in small numbers.

Although spiral and sheath nematodes were encountered in healthy and diseased stands, the data suggested that they were twice as abundant in diseased as in healthy stands. The American dagger nematode was found as frequently in diseased as in healthy stands, with no apparent quantitative differences. Populations of lance nematodes were generally higher in healthy stands.

It is possible that certain plant-parasitic nematodes are important factors contributing to the over-all root damage involved in the littleleaf complex. Further research is needed on the relationships of nematodes to shortleaf pine to determine their capacity for causing intensive root damage under forest conditions.

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[2] Personal correspondence on file with the author at Athens, Georgia.

Table 1. Nematodes per pint of soil in littleleaf and healthy shortleaf pine stands of the Piedmont.

Sample location	Xiphinema		Hemicycliophora		Helicotylenchus[b]		Hoplolaimus	
	LL	H	LL	H	LL	H	LL	H
Franklin Co., Ga.	132.6[a]	183.0	-	30.2	27.6	42.8	7.6	-
Jones Co., Ga.	35.2	152.8	217.8	160.2	292.6	345.4	-	-
Hall Co., Ga.	82.8	20.2	160.2	67.8	15.2	-	-	70.0
Elbert Co., Ga.	135.2	95.2	57.6	50.2	15.2	202.8	17.6	-
Union Co., S. C.	32.6	110.2	17.6	45.2	80.2	-	-	-
Union Co., S. C.	117.6	27.6	57.6	30.2	97.6	42.8	-	52.8
Davidson Co., N. C.	157.8	50.2	155.4	17.6	692.8	5.0	67.6	-
Forsyth Co., N. C.	100.2	70.2	25.2	77.6	112.8	115.2	7.6	97.8
Buckingham Co., Va.	-	90.2	47.6	137.8	1557.8	57.6	40.2	-
Appomattox Co., Va.	2.6	85.2	20.2	35.2	22.8	40.2	2.6	47.6
Harris Co., Ga.	42.8	22.6	75.2	50.2	50.2	-	7.6	-
Cleburne Co., Ala.	7.6	12.8	158.0	15.0	42.6	92.8	-	55.2
Mean[c]	77.0	76.7	90.2	59.8	250.6	105.0	21.5	64.7

[a] Average of 5 samples

[b] Both species of Helicotylenchus were counted together because of mixed populations within stands.

[c] Based upon 5 to 12 sample locations as indicated.

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PATHOGENICITY OF PRATYLENCHUS VULNUS ON BOXWOOD, BUXUS SEMPERVIRENS VAR. ARBORESCENS[1, 2]

W. W. Osborne and W. R. Jenkins

Abstract

Inoculation of uniform, 6-month-old rooted boxwood cuttings with 2000 *Pratylenchus vulnus* resulted in retarded plant growth and a bronzing of the foliage after 318 days. The average increase in fresh weight of inoculated plants was 8.7 g, while uninoculated plants weighed an average of 29.8 g. The nematode population increased to 27,230 per plant during this period. Destruction of tissue was extensive with large numbers of adults, larvae, and eggs observed in the infected cortex. It was concluded that *P. vulnus* is an important pathogen of boxwood.

INTRODUCTION

Boxwood has been included as a host of several nematodes, including *Pratylenchus vulnus* Allen & Jensen (3, 4, 5). Others (1, 6, 7, 8, 9) have indicated that boxwood is attacked by members of the genus *Pratylenchus*, without reference to the individual species involved. The pathogenic potential of this nematode, however, has not been demonstrated. The purpose of the research reported here was to establish the pathogenicity of *P. vulnus* to boxwood, *Buxus sempervirens* var. *arborescens*.

MATERIALS AND METHODS

Twenty-four 6-month-old rooted cuttings of boxwood were paired according to weight, and potted in 6-inch azalea pans in a steamed sandy loam soil. One member of each pair was inoculated with 2000 *P. vulnus* collected from greenhouse cultures maintained on boxwood. Recovery of nematodes from infected roots for use as inoculum was accomplished by placing about 1 kg of infected roots in water in a 2-inch glass cylinder. A stream of air was introduced through a tube at the bottom of the cylinder to aerate the system. Nematodes which emerged from the roots were collected on a 325-mesh sieve and rinsed several times with tap water.

Plants were inoculated by pouring 20 ml of water containing 2000 nematodes over the bared roots of the cuttings at time of potting. Control plants received 20 ml of tap water.

All plants were fertilized at 3-week intervals with a water-soluble fertilizer having a 7-6-19 analysis. Subsequently, they were maintained under greenhouse conditions for 318 days, during which time daytime temperatures did not exceed 90°F and night temperatures ranged between 70° and 75°.

Internal injury to roots by *P. vulnus* was studied by standard histological methods. Infected and non-infected roots were fixed in Randolph's Modified Navashin Fluid, dehydrated through a tertiary butyl alcohol series, and embedded in Fisher's "Tissuemat." Sections were cut on a rotary microtome 10 μ thick, fixed to slides with Haupt's adhesive, stained with safranin-fast green, and mounted in Fisher's "Permount."

FIGURE 1. A comparison between boxwood plants (*Buxus sempervirens* var. *arborescens*) 318 days after inoculation with 2000 *Pratylenchus vulnus* (right) and uninoculated.

FIGURE 2. Cross section of a boxwood root infected with *Pratylenchus vulnus*. Note extensive cortical damage and reduction in xylem elements near infection sites.

FIGURE 3. Longitudinal section of a boxwood root infected with *Pratylenchus vulnus*. Note colonization in cortical parenchyma with resultant cavity formation.

FIGURE 4. Cross section of an uninfected boxwood root.

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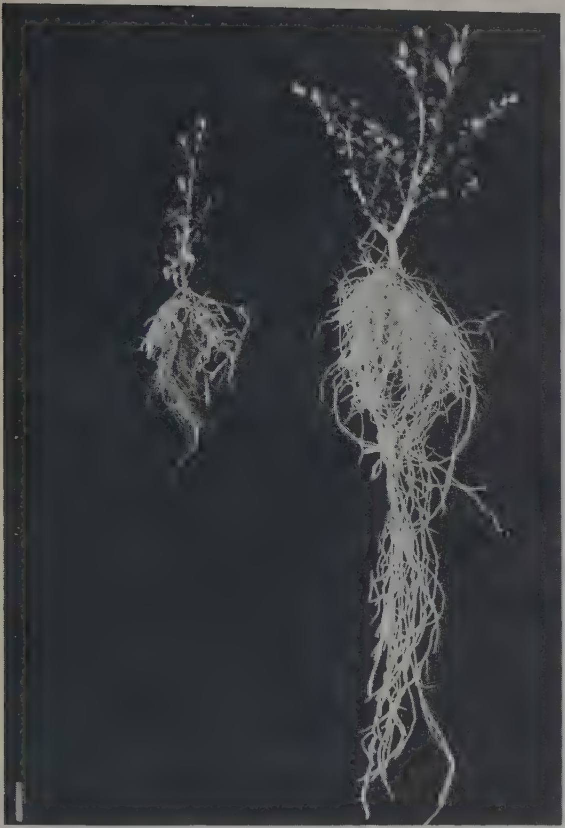
[2] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University.



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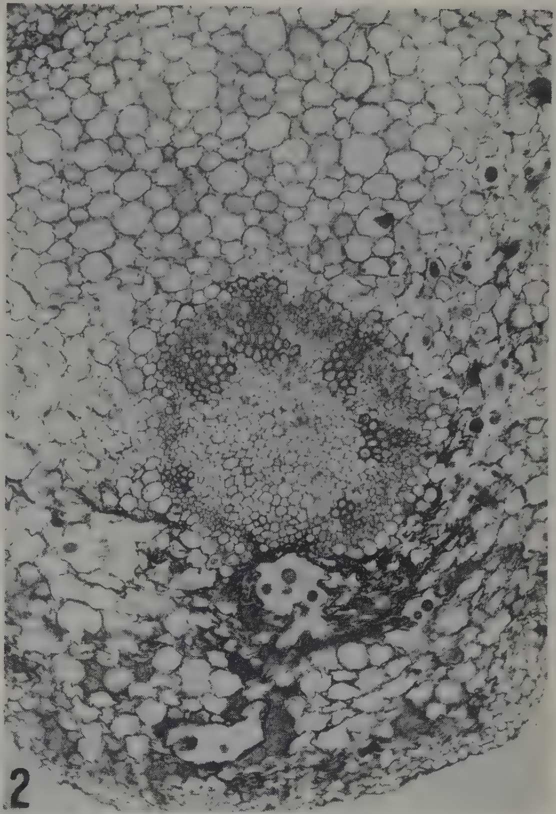

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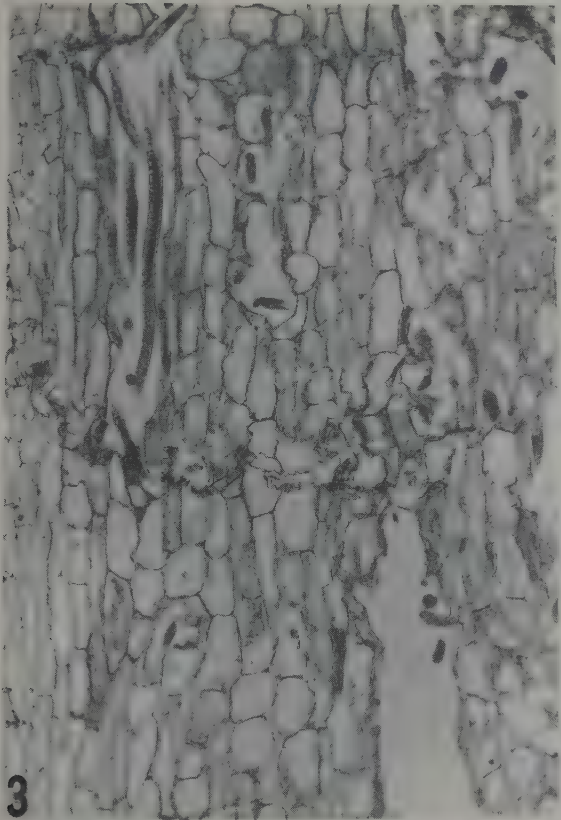

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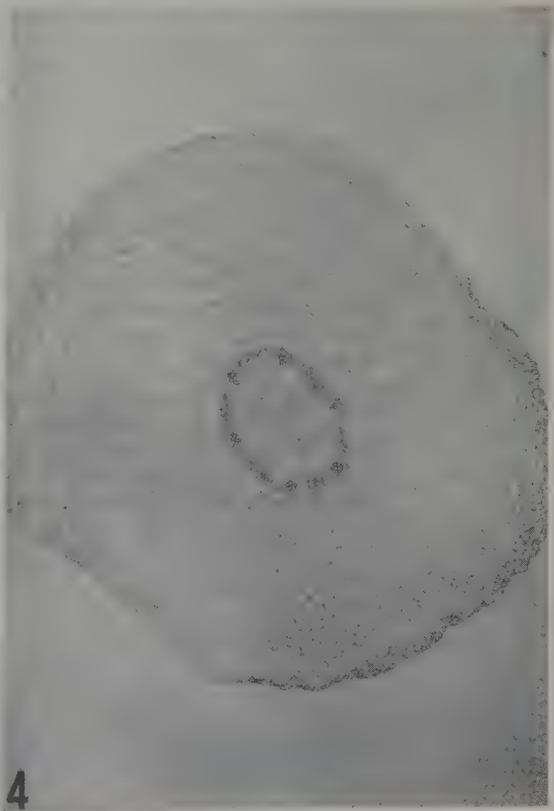

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RESULTS AND DISCUSSION

Boxwood plants infected with P. vulnus were severely retarded in growth (Fig. 1) and exhibited extensive necrosis of individual roots. Owing to this injury, the entire root system became discolored and brown in appearance, in comparison with uninfected control plants. A bronzing of the foliage similar to that caused by Helicotylenchus buxophilus (2) was characteristic on infected plants.

The average increase in fresh weight of control plants after 318 days was 29.8 g, while infected plants gained an average of only 8.7 g. The population of P. vulnus increased from the initial 2000 per plant to an average of 27,230 per plant during the test period. No stylet-bearing nematodes were recovered from the roots and soil of control plants.

Examination of sectioned and stained roots from infected plants revealed adults, larvae, and eggs in the cortex. No nematodes were observed in the epidermal or stelar region. Xylem elements were less numerous in the stelar region when the surrounding cortical tissue was severely damaged (Fig. 2).

Extensive nematode colonization of cortical parenchyma resulted in the formation of cavities (Figs. 2 and 3), a common symptom associated with parasitism by members of the genus Pratylenchus. Many cells in infected roots contained green-stained granular material while other cells were devoid of cytoplasm and their cell walls stained a deep red. This reaction was interpreted as an indication of necrosis. A section of a control root is illustrated in Figure 4.

The extensive destruction of root cortical tissue in boxwood by P. vulnus adversely affects the normal functions of this tissue and results in a devitalized root system and a stunted, nonvigorous plant.

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RECORDED DUTCH ELM DISEASE DISTRIBUTION IN NORTH AMERICA AS OF 1961[1]

x noted for map 30

Francis W. Holmes[2]

During 1960 and 1961 Ceratocystis ulmi (Buisman) Moreau, the pathogen of the Dutch elm disease, was recorded for the first time in four additional States: Arkansas (39), Minnesota (31), Nebraska (35), and Oklahoma (32). It was newly recorded also within previously infested States and Provinces in many localities not plotted on the map published as of 1959 (20). A revision of the Dutch elm disease map accordingly is presented in Figure 1, as of 1961.

This year the checkerboard pattern, which formerly indicated records available only on a province-wide or state-wide basis, has been omitted. Wherever numerous individual town, township, or county records are scattered throughout a State or Province or occur along the border of an adjoining State or Province, the reader is justified in supposing that the Dutch elm disease probably occurs in most or all intervening or nearby adjoining areas where elms grow.

It should be borne in mind again that this map does not purport to show where the Dutch elm disease is currently rife, but only where the presence of the causal fungus has been confirmed at one time or another in the past. An example of this distinction is in Colorado, where the Dutch elm disease was found in several communities in 1948 but where it has not been found in recent years.

Numerous inquiries have been received in response to the publication of maps of North American distribution of the Dutch elm disease recorded as of 1955 (17), 1956 (18), 1957 (19), and 1959 (20). As a result, data have been compiled, largely by reliance upon correspondents who have also provided the fungus-distribution records, to show for each infested State and Province the year of first confirmation of the Dutch elm disease and the earliest publication reporting that new record. This information is presented chronologically and alphabetically by States in Table 1.

In some cases the earliest published mention of the presence of the Dutch elm disease in a given State or Province is not the one that specifies when the disease was first found there. In other cases several publications appeared at approximately the same time. In such instances, two or more citations are given. Earlier reports may exist than those here cited. The author would appreciate having these called to his attention.

Table 1. Year of first discovery and citation to first report of the Dutch elm disease in each State and Province in North America through 1961.

Chronological	Alphabetical
1930 - Ohio (21, 22, 23)	Arkansas - 1961 (39)
1933 - Connecticut (13, 15)	Colorado - 1948 (16, 33)
1933 - Maryland (15, 24)	Connecticut - 1933 (13, 15)
1933 - New Jersey (15, 37, 38)	Delaware - 1944 (4)
1933 - New York (38)	District of Columbia - 1947 (5, 36)
1934 - Indiana (3, 40)	Illinois - 1950 (11)
1934 - Virginia (10)	Indiana - 1934 (3, 40)
1937 - West Virginia (3, 30a)	Iowa - 1957 (7)
1938 - Pennsylvania (3, 30a)	Kansas - 1957 (27, 34)
1941 - Massachusetts (26)	Kentucky - 1945 (4)
1944 - Delaware (4)	Maine - 1952 (28)
1944 - Quebec (29)	Maryland - 1933 (15, 24)
1945 - Kentucky (4)	Massachusetts - 1941 (26)
1945 - Vermont (9)	Michigan - 1950 (1)
1946 - Rhode Island (5, 6)	Minnesota - 1961 (31)
1946 - Tennessee (2, 30)	Missouri - 1952 (27, 34)
1946 - Ontario (25)	Nebraska - 1960 (35)
1947 - District of Columbia (5, 36)	New Brunswick - 1957 (8)
1948 - Colorado (16, 33)	New Hampshire - 1950 (14)
1950 - Illinois (11)	New Jersey - 1933 (15, 37, 38)
1950 - Michigan (1)	New York - 1933 (38)

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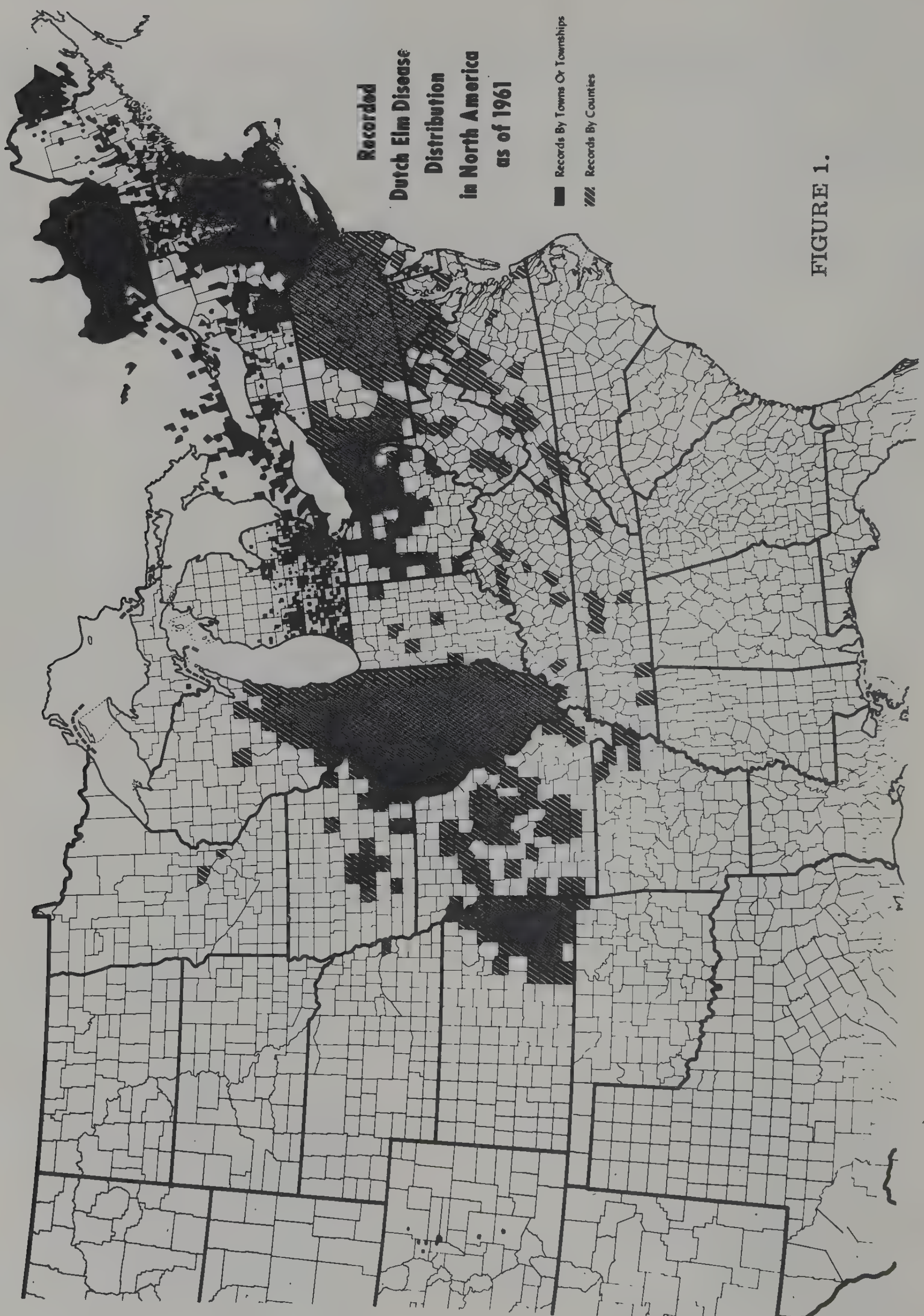
Recorded
Dutch Elm Disease
Distribution
in North America
as of 1961
■ Records By Towns Or Townships
▨ Records By Counties

FIGURE 1.

Table 1. (continued)

Chronological	Alphabetical
1950 - New Hampshire (14)	Ohio - 1930 (21, 22, 23)
1952 - Maine (28)	Oklahoma - 1961 (32)
1952 - Missouri (27, 34)	Ontario - 1946 (25)
1956 - Wisconsin (12)	Pennsylvania - 1938 (3, 30a)
1957 - Iowa (7)	Quebec - 1944 (29)
1957 - Kansas (27, 34)	Rhode Island - 1946 (5, 6)
1957 - New Brunswick (8)	Tennessee - 1946 (2, 30)
1960 - Nebraska (35)	Vermont - 1945 (9)
1961 - Arkansas (39)	Virginia - 1934 (10)
1961 - Minnesota (31)	West Virginia - 1937 (3, 30a)
1961 - Oklahoma (32)	Wisconsin - 1956 (12)

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POSTHARVEST DETERIORATION OF THE DOWNY MILDEW-SUSCEPTIBLE LETTUCE VARIETY GREAT LAKES AND THE RESISTANT VARIETY CALMAR

F. W. Zink and J. E. Welch[1]

Summary

In the susceptible Great Lakes variety of lettuce, chlorotic lesions on the wrapper leaves retained at harvest showed growth and sporulation of the downy mildew fungus (Bremia lactucae Regel) on both the lower and upper surfaces after an 8-day storage period at 40° F. No fungus growth appeared on the resistant Calmar variety. The outermost head leaf of all Great Lakes plants studied appeared healthy at time of harvest but after the 8-day 40° storage period, a high percentage of these leaves exhibited fungus growth and sporulation. Infected tissues were invaded by unidentified bacteria and fungi and the secondary rots that resulted were a major factor in the decrease in visual quality of downy mildew-infected heads.

There were no significant differences in the rate of deterioration in visual quality of Great Lakes with no apparent downy mildew infection and Calmar. Therefore, the variations in quality ratings reported here resulted from downy mildew infection and were not due to some inherent factor, or factors, affecting the postharvest physiology of Calmar.

On eastern terminal markets downy mildew may be a serious disease on California-grown lettuce (3). The development of resistant Calmar (1) provided a method for comparing the rate of postharvest deterioration of a susceptible infected variety with a resistant variety both grown, harvested, and stored under identical conditions.

Calmar is a crisphead variety of the Great Lakes type. Downy mildew resistance in Calmar is conferred by a single dominant gene that was originally found in PI 104854 (1, 2, 4).

The leaves of a market-mature plant of head lettuce range through a wide variety of sizes and shapes. The outermost leaves are termed "frame" leaves, and the few intermediate leaves that fold loosely over the head are called "wrapper" leaves. The inner leaves are interlapped to form the firm terminal bud that is commonly called the head. The leaves forming the terminal bud are called "head" leaves, and the outermost leaf that covers the head is termed the "cap" leaf.

The purpose of this study was to determine the effect of downy mildew on the rate of postharvest deterioration and its relationship to subsequent invasion of secondary slime- and rot-producing organisms.

MATERIALS AND METHODS

Great Lakes and Calmar lettuce was harvested from adjacent beds in nine commercial fields in the Central Coastal district of California during the 1961 season. Each treatment consisted of ten comparable heads of Great Lakes and Calmar at market maturity which were free from physical defects and diseases other than downy mildew in Great Lakes. Downy mildew symptoms on the wrapper leaves retained on the Great Lakes heads chosen for storage tests ranged from pale green spots on the upper sides of affected leaves to moderately chlorotic angular lesions possessing sporulation on the lower surfaces. Care was taken to select Great Lakes heads that showed no symptoms of downy mildew infection on the cap leaf. The severity of downy mildew infection in the several fields was variable and was recorded for each trial.

The heads were harvested, trimmed to four wrapper leaves, packed carefully to avoid bruising, vacuum cooled, and stored 8 days at 40° F. On the eighth day each head was given a quality rating, and then trimmed to the cap leaf and given a second quality rating. The samples were then transferred to 70°, examined daily, and held until judged unsalable. The procedure was intended to simulate an 8-day transit period from California to New York and a subsequent interval at the retail level.

To evaluate quality loss in terms of appearance, each head was individually rated according to the following scheme: 9 = excellent -- heads freshly harvested, free from all defects,

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Table 1. Effect of downy mildew on the visual quality of lettuce held 8 days after harvest at 40° F.

Trial number	Variety	Downy mildew rating in field	Quality rating[a] At time of harvest after heads trimmed to 4 wrapper leaves[b]	After 8 days at 40° F before additional trimming	After 8 days at 40° F after trimming to cap leaf[c]
1	Great Lakes 118	none	9.0	7.4	8.0
	Calmar	none	9.0	7.3	7.9
2	Great Lakes 118	none	9.0	7.5	8.0
	Calmar	none	9.0	7.4	8.0
3	Great Lakes R-200	none	9.0	7.2	7.6
	Calmar	none	9.0	7.1	7.6
4	Great Lakes 118	moderate	7.6	5.1	6.2
	Calmar	none	9.0	7.1	7.9
5	Great Lakes 65	moderate	7.3	5.7	6.5
	Calmar	none	9.0	7.0	7.9
6	Great Lakes 366	moderate	7.5	5.3	6.4
	Calmar	none	9.0	6.9	7.5
7	Great Lakes 366	severe	6.9	4.0	5.7
	Calmar	none	9.0	7.3	8.0
8	Great Lakes 118	severe	6.7	3.6	5.5
	Calmar	none	9.0	7.0	7.6
9	Great Lakes 66	severe	7.0	4.7	5.8
	Calmar	none	9.0	7.3	7.9

[a] Mean quality rating of 10 heads.
[b] Wrapper leaves are the leaves that fold loosely over the head.
[c] The cap leaf is the outermost leaf of the head.

no downy mildew symptoms; 7 = good -- only a few minor defects, or trace to slight downy mildew infection, or both; 5 = fair -- few defects, or moderate downy mildew infection, or both; and 3 = unsalable -- some defects, or severe downy mildew infection, or both.

RESULTS AND DISCUSSION

Table 1 presents the mean quality rating of the lettuce at the time of harvest after the heads were trimmed to four wrapper leaves and following an 8-day holding period at 40° F. Lettuce trimmed to four wrapper leaves represents the appearance of the heads on arrival at an eastern terminal market, and heads trimmed to the cap leaf represent the appearance after preparation for retail display. Trials 1, 2, and 3, in which there was no apparent downy mildew infection, showed no significant differences between Great Lakes and Calmar in visual quality ratings. However, the quality ratings of Great Lakes infected with downy mildew in trials 4 through 9 were significantly lower than Calmar. Great Lakes samples from trials in which downy mildew infection was rated severe had a consistently lower quality rating than those trials in which infection was rated moderate. Considerable growth and sporulation of downy mildew were present in infected samples after the 8-day holding period at 40°. During the simulated transit period, the older lesions on the wrapper leaves turned brown and became soft and slimy as secondary decay organisms gained entrance through the tissues infected with the downy mildew fungus.

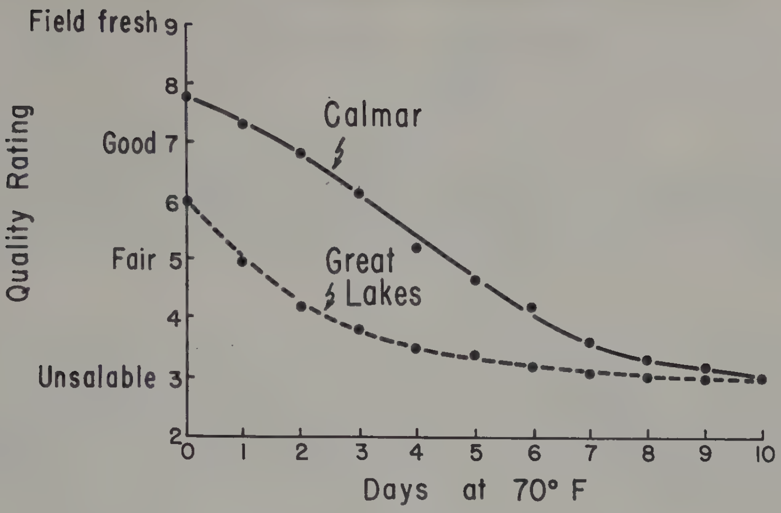

FIGURE 1. Visual quality of downy mildew-infected Great Lakes and resistant Calmar at daily intervals during a 70° F simulated retail-holding period which followed an 8-day 40° simulated transit period.

Figure 1 shows loss in visual quality during the 70° F simulated retail-holding period which followed the 8-day 40° simulated transit period. The heads were placed in 70° storage after they had been trimmed to the cap leaf. The curves represent the best fit for the average quality ratings given 60 heads of Great Lakes and 60 heads of Calmar in trials 4 through 9. The lower quality rating of the samples of Great Lakes at the beginning of the 70° holding period was due to downy mildew lesions on the cap leaf and the subsequent development of rots. A high percentage of the Great Lakes heads had developed fungus growth and sporulation on the cap leaf during the preceding 8-day holding period at 40°. Infection of the cap leaf probably occurred in the field but no symptoms were apparent at harvest. Secondary rots that developed during the simulated transit and retail-holding periods were a major factor in the deterioration in visual quality of downy mildew-infected heads.

Ramsey, et al. (3) reported that when downy mildew is prevalent in the field and transit temperatures are above 40° F, considerable infection may be evident at terminal markets on California-grown lettuce. Best control of downy mildew after harvest has been obtained by removing the older wrapper leaves, rapid precooling after packing, and transporting at temperatures as close to 32° F as possible. The introduction of downy mildew-resistant varieties should improve the general appearance of lettuce and reduce losses caused by secondary decay organisms.

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ROSE AS A SOURCE OF PRUNUS [Peach] RINGSPOT VIRUS[1]

H. C. Kirkpatrick, R. C. Lindner, P. W. Cheney and S. O. Graham[2]

Summary

Of 32 samples of rose mosaic from Washington, 14 or 44% were mechanically transferable to herbaceous hosts. Twelve of the 14 isolates were identical to Prunus ringspot virus in herbaceous host range, symptom syndrome, electrophoretic mobility, Mahaleb, Montmorency and Shirofugen indexing.

INTRODUCTION

Ringspot virus (RSV) of stone fruits has been known to occur naturally in rose. Cochran (1) reported 2 of 11 California rose varieties to be positive for RSV when indexed to peach.

Gilmer (3) compared a rose isolate with a number of stone fruit isolates inoculated mechanically to cucumber. In Italy, Gualaccini (4) reported a rose with mosaic symptoms that killed 'Kwanzan' cherry when chip budded to it. Fulton's (2) mechanically transmissible rose mosaic has many of the characteristics of some forms of RSV. This report concerns RSV and other viruses recovered from various roses and rose understocks showing mosaic patterns in Washington.

MATERIALS AND METHODS

In 1957, 28 samples of rose and rose understocks with mosaic-type symptoms were obtained from commercial nurseries. Four other samples with mosaic symptoms from home plantings were added. Leaf tissue was inoculated to cucumber by using Yarwood's cut-leaf technique (6) and 14 of the 32 samples were infective. The 14 isolates have been tested in varying degrees to a host range selected for determination of RSV and prune dwarf virus (PDV). The isolates in cucumber were mechanically inoculated to herbaceous species and to Prunus mahaleb or 'Lovell' peach seedlings for graft transmission to a stone fruit host range.

Isolates selected on the basis of host-range reaction were studied further after partial purification from cucumber by methods previously reported (5).

RESULTS

Twelve of the 14 isolates had a host range and symptom syndrome typical of RSV. The other two isolates, which were different from each other and from RSV, are not considered in this report (Table 1).

Two of the isolates, YRM and RCL, were partially purified and compared with RSV electrophoretically. All three isolates have a mobility of 6.7 x 10.5.

The few attempts to transfer the rose isolates and RSV mechanically from herbaceous hosts to rose have been unsuccessful, so it is not known whether the mosaic patterns in rose are caused by RSV. However, since our sampling indicates 38% RSV, apparently stone fruit ringspot is present in commercial and home rose plantings.

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[1] Scientific Paper No. 2259. Washington Agricultural Experiment Station, Pullman, Washington. Project No. 865.

[2] Respectively, first and third authors Pathologists, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Wenatchee, Washington; second and fourth authors Plant Pathologist and Associate Plant Pathologist, Washington Agricultural Experiment Stations.

Table 1. Host range to compare rose virus isolates with Prunus ringspot virus.

	Isolates				
	RSV	YRM	RCL	C, D, J, G	HY 1, 2, 8, 12, 14, 16
Chenopodiaceae					
Chenopodium amaranticolor	SR	SR	SR		
Cucurbitaceae					
Cucumis sativus 'Chicago Pickling'	SM	SM	SM	SM	SM
Cucurbita maxima 'Buttercup'	C or NLL	NLL	NLL	NLL	NLL
Leguminosae					
Sesbania exaltata	NLL	NLL		NLL	
Cyamopsis tetragonoloba	NLL	NLL	NLL	NLL	NLL
Solanaceae					
Nicotiana tabacum	H	H	H	H	
Rosaceae					
Prunus mahaleb	SCM	SCM	SCM		
Prunus serrulata 'Shirofugen'	N	N	N		
Prunus cerasus 'Montmorency'	SNR	SNR			

C = chlorotic
LL = local lesion
N = necrotic
S = systemic
H = symptomless
M = mottle
R = ringspot

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THE EFFECT OF POWDERY MILDEW ON THE GROWTH OF KENTUCKY BLUEGRASS

T. A. Gaskin and M. P. Britton[1]

Powdery mildew caused by Erysiphe graminis DC. has been generally considered a disease of minor importance on Kentucky bluegrass (Poa pratensis) (1, 2). Observations made over a period of years in Illinois indicate that this disease might be an important factor in the deterioration of bluegrass lawns in heavily shaded areas. This is especially true with the Merion selection of Kentucky bluegrass which is very susceptible to powdery mildew. The pathogen may become established on Merion bluegrass in late April and continue to spread throughout the summer and fall. First infections are usually observed near the north and east side of buildings and under dense shrubbery. In the fall, infection may be extensive enough to impart a whitish cast to densely shaded Merion bluegrass lawns.

This study was conducted to determine growth reduction caused by E. graminis of Kentucky bluegrass growing in sunlight and in shade.

Sod of Merion bluegrass was transplanted on February 1, 1962 into four 1-foot square flats and grown in the greenhouse. Two flats were placed under Lumite shade cloth providing 66% shade and the other two were unshaded. One flat in each treatment was inoculated with the pathogen and the other sprayed at weekly intervals with Karathane WD (dinitro (1-methyl heptyl) phenyl crotonate 22.5%, other nitro phenols 2.5%) at a rate of 1 level teaspoonful per gallon of water. Uniformly heavy infection developed in the inoculated flats but no powdery mildew developed in those that were sprayed with Karathane. The grass in all flats was clipped periodically at a height of 3/4 inch above the soil line. All clippings from each flat were collected, air dried, and weighed to determine differences in growth. At the termination of the study, the plants in four circular sod pieces 4 inches in diameter were removed from each flat. The soil was gently washed from the roots and air-dry weights of roots and tops were obtained separately.

Shading in the absence of powdery mildew has a depressing effect on the growth of Kentucky bluegrass (Tables 1 and 2). With one exception the amount of leaf growth of disease-free plants in the sun varied directly with the percentage of possible sunlight. The growth rate of leaves under shade remained fairly constant in each period regardless of the amount of sunlight available.

Table 1. Dry weight of leaf clippings from Merion bluegrass grown in sunlight or shade with and without mildew infection.

Growth period between clippings	% possible sunshine during growth period[a]	Dry weight of leaf clippings (in grams)			
		Mildew		No Mildew	
		sun	shade	sun	shade
2/1/62 to 2/16/62	28	0.76	0.34	1.15	0.30
2/17/62 to 2/27/62	9	1.00	0.01	2.00	0.36
2/28/62 to 3/9/62	42	0.50	Dead	2.66	0.44
3/10/62 to 3/21/62	33	0.48	----	1.91	0.36
3/22/62 to 4/3/62	69	0.60	----	3.31	0.21

[a] U. S. Weather Bureau.

Table 2. Dry weight of tops, roots and rhizomes, and the total plant weight of Merion bluegrass grown in sunlight or shade with and without powdery mildew for 62 days.

Plant part	Av. dry weight of total plant parts in 4-inch diameter sod piece (in grams)			
	Mildew		No Mildew	
	sun	shade	sun	shade
Tops (leaves and sheaths)	1.0	0	2.2	0.5
Roots and rhizomes	1.1	0	3.2	0.9
Total plant	2.1	0	5.4	1.4

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[1] Respectively, Research Director and Plant Pathologist, Warren's Turf Nursery, Palos Park, Illinois and Assistant Professor of Plant Pathology, University of Illinois.

Table 3. Effect of powdery mildew on the number and length of rhizomes of common Kentucky bluegrass.

Criteria for evaluation	Mildew infected (121 plants)	No mildew (205 plants)	% reduction from non-infected plants
Average number rhizomes per plant	3.6	3.9	7.6
Average total length of rhizomes per plant	24.0 cm	31.0 cm	22.6[a]

[a] Significant difference at the 5% level.

A further reduction of leaf growth was noted in plants infected with powdery mildew. The reduction was much greater in the shaded treatments and in approximately 5 weeks resulted in the death of the plants. Infected plants receiving sunlight were not killed during the test period, but much of the leaf tissue was destroyed.

The total weight of the top growth and roots at the termination of the study was similarly reduced in shade without mildew infection and in sunlight with mildew infection (Table 2).

To determine the effect of powdery mildew on rhizome growth, individual tillers of common Kentucky bluegrass were established in flats on January 17, 1962. These were grown in the greenhouse without shading and clipped regularly to a 1-inch height until May 1 when the length and number of rhizomes were recorded. A heavy infection of powdery mildew developed on 121 of 326 plants; the rest were free from disease (Table 3). There was a significant reduction in the average length of rhizomes on the mildew-infected plants but not in the number of rhizomes per plant.

The results of these studies indicate that powdery mildew may cause a significant reduction in the growth of Kentucky bluegrass. Severe damage and death of infected plants occurs in dense shade. The damage is expressed by a reduction in growth of leaves, roots and rhizomes of the plants. A similar growth reduction, but no killing of plants, results when non-infected plants are subjected to dense shade.

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PARTICULARLY SIGNIFICANT SPECIMENS OF ELSINOË AND SPHACELOMA TAKEN BY U. S. PLANT QUARANTINE INSPECTORS

Alice J. Watson and Anna E. Jenkins[1]

We are continuing our collaboration with the Plant Quarantine Division of the United States Department of Agriculture by giving specific taxonomic attention to certain fungus interceptions made by inspectors during their regular activities. Such interceptions come from foreign cargo, mail, baggage, and various means of transportation. They are also taken in surveys made in port areas and in Puerto Rico.

Under the "Remarks" column in Table 1, we have called attention to a few outstanding discoveries of various specimens. Some of these discoveries established new distribution records.

We have not cited previously known distribution.

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[1] Plant Quarantine Division and Crops Research Division, respectively, United States Department of Agriculture.

Table 1. Port interceptions including specimens collected in port areas, U. S. and Puerto Rico.

Species	Host	Origin	Port number	Date	Inspector	Remarks
Elsinoë australis Bitanc. & Jenkins (Mycologia 28: 491. 1936)	Citrus sinensis	Brazil	Philadelphia 47882	Oct. 12 1960	I. P. Boeshore	On these four specimens (rind of ripe fruit) perfect stage of Elsinoë copiously present in slides made by E. T. Ozaki, an outstanding discovery. There are only two other records of this stage, the first from the state of Saõ Paulo, Brazil (1), the other Rio Grande do Sul, Brazil (2).
	do.	do.	Philadelphia 48056	June 16 1961	N. E. Arehart	
	do.	do.	New York 167729	June 14 1961	E. B. Lee	
	do.	do.	New York 169853	June 6 1961	R. Kahan	
Elsinoë lepagei Bitanc. & Jenkins (Arq. Inst. Biol. 12: 5. 1941, described on Achras sapota)	Pouteria campechiana	Mexico	Nogales 83159	Jan. 26 1961	D. E. Noel	New area of distribution, previously reported on P. campechiana (3).
Elsinoë piri (Wor.) Jenkins (J. Agr. Research 44: 696. 1932) conidial stage, Sphaceloma pirinum (Pegl.) Jenkins (Phytopathology 36: 460. 1946)	Malus sylvestris	USSR	New York 99863	June 4 1947	L. J. Burke	These interceptions, together with those previously noted by Jenkins (4, 6) are sufficient in themselves to reveal the wide European distribution of E. piri. The record from Germany is tentative, requiring further substantiation. No other available record from that source[a].
	do.	Poland	New York 10372	Aug. 15 1948	C. S. Tuthill	
	do.	Spain	New Orleans 37531	Nov. 11 1961	A. A. Ayson	
	do.	Germany	Miami 9029	Apr. 3 1960	B. E. Davis	
Sphaceloma murrayae Godsinsky & Jenkins (Rev. Argentina Agron. 10: 56. Mar. 15, 1943)	Salix nigra	Texas	Roma 443	Mar. 16 1945	A. A. Stalmach	New State record. Identification of S. murrayae on willow (Salix lasiandra) from Skagit Co., Washington, Aug. 1943, is based on an Inspectors collection (5).
Sphaceloma poinsettiae Ruehle & Jenkins (Proc. Biol. Soc. Wash. 55: 83. 1942)	Euphorbia pulcherrima	X Arizona	Douglas 15456	Sept. 17 1957	J. H. Russell	New State records.
	do.	Florida	Miami 10315	Dec. 5 1959	H. L. Rubin	This species described from Florida and Hawaii.
slip done map 34	do.	X Texas	Brownsville 82238	Nov. 10 1960	E. C. Heinrich	New State record.
	do.	Puerto Rico	San Juan 14949	Sept. 21 1960	H. L. Rubin	As reported by Rubin (9), who cites an earlier record from Jamaica, and first discovery in Puerto Rico.
Sphaceloma punicae Bitanc. & Jenkins (Proc. Am. Sci. Cong. 8th, Washington, D. C. 1940, 3: 163. 1942)	Punica granatum	Texas	Laredo 53028	July 29 1952	A. H. Lewis	New State record. Heavy infection on one tree. Many interceptions from Mexico since our report of 1951 (8).
	do.	Dominican Republic	New York 174748	Feb. 23 1962	K. Shiroishi	First West Indian report.
Sphaceloma spondiadis Bitanc. & Jenkins (Proc. Am. Sci. Cong. 8th, Washington D. C. 1940, 3: 161. 1942)	Spondias cytherea "vi-apple"	British Guiana.	Miami 9892	Sept. 26 1959	R. Faircloth	All three interceptions represent new areas of distribution with a new host species in the case of S. lutea.
	do.	Puerto Rico	New York 169478	Apr. 25 1961	D. Shiroma and S. Cousins	
	Spondias lutea "jobo"	Barbados	San Juan 11866	Oct. 20 1954	L. T. Sanders	
Sphaceloma zorniae Bitanc. & Jenkins (Arq. Inst. Biol. 11: 47. 1940), described on Zornia diphylla)	Zornia reticulata	St. Kitts Leeward Islands	San Juan 11535	Mar. 4 1954	A. A. Ayson	Second record, representing new distribution and a new host species.

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[a] Records of E. piri in the United States (Washington, Oregon) based on surveys by Plant Quarantine Inspectors (7).

A SEVERE OUTBREAK OF SELENOPHOMA SPOT ON 'GAINES' WHEAT[1]

C. S. Holton and Laurence H. Purdy[2]

A severe outbreak of Selenophoma spot (Selenophoma donacis (Pass.) Sprague & A. G. Johns.[3]) occurred in several seed-increase fields of the newly-released semidwarf wheat variety 'Gaines' in the Pacific Northwest in 1962. During June the disease was observed in every field of this variety examined in Washington, Oregon, and Idaho. There was a wide range in prevalence and severity, which apparently depended on the degree of vegetative development of the plants. The majority of fields examined had relatively thin stands and were only mildly infested. Others (the ones with the heaviest stands of vigorously developed plants) were heavily infested. In one 50-acre field near Pullman, Washington virtually 100% of the upper three leaves of the plants were infected and the great majority were damaged severely, as indicated by extensive irregular areas of necrotic spotting (Fig. 1).

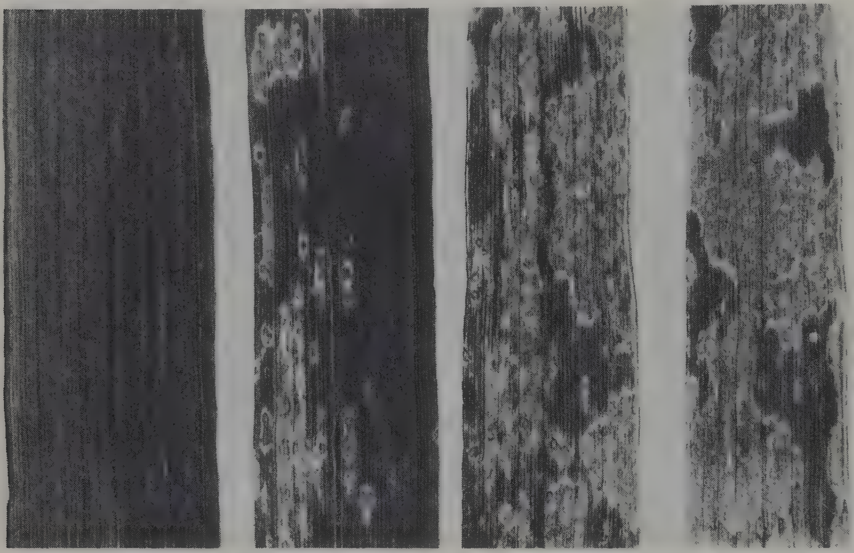

FIGURE 1. Portions of four leaves of 'Gaines' wheat showing one healthy leaf (left) and three diseased leaves exhibiting different degrees of damage from Selenophoma spot.

Selenophoma spot was identified by Sprague[4] in 1948 as a new wheat disease for North America. He observed it in a geographic range extending from the Thatuna Hills near Moscow, Idaho, west to Central (Ferry) Bridge, Washington, including variety plots of the Washington Agricultural Experiment Station, where it was serious enough to attract the attention of wheat breeders. Sprague[4] recorded the differential reaction of varieties in the experimental plots, and noted that Orfed and Rex were highly susceptible and several others, including Hymar x Elgin[3] (later named Elmar), were highly resistant.

Historically, Selenophoma spot has been of little or no consequence as a disease of wheat in the Pacific Northwest. It was moderately severe in commercial fields of 'Golden' in 1948 but apparently has been largely obscure since then. The sudden outbreak on 'Gaines' in 1962 was spectacular not only in its severity but also in its obvious preference for this variety. Other varieties within the area of infestation, primarily 'Brevor', 'Omar', and 'Burt', were mainly free from the disease. These observations suggest that Selenophoma spot possibly might develop into a disease problem of major importance on 'Gaines' wheat when this variety comes into full-scale production.

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[2] Pathologists, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Pullman, Washington.

[3] Sprague, R. 1950. Diseases of Cereals and Grasses in North America. The Ronald Press Co., New York. 538 pp.

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STAGONOSPORA BROMI ON BROMUS INERMIS

K. E. Zeiders and J. H. Graham[1]

Abstract

Leaf lesions caused by Stagonospora bromi are distinguished from similar leaf spots of Bromus inermis by the light central area and the pycnidia in the necrotic tissue. The fungus fruits abundantly on lima bean agar. The optimum temperature for mycelial growth and spore germination in culture was near 25° C. Pycnidia formed in 3 days at 20°, 25°, and 30°, in 4 days at 15°, and in 11 days at 10° C. Spore discharge from pycnidia was highest at 20°. No consistent effect of previous incubation temperature upon spore discharge was detected. In cultural studies no Phyllosticta stage was observed to be associated with S. bromi. In artificial inoculations of B. inermis, the optimum temperature for infection and disease development was between 20° and 24°. Young plants of Dactylis glomerata, Phleum pratense, Festuca elatior, F. arundinacea, Agrostis alba, Lolium perenne, Phalaris arundinacea, and Triticum vulgare were nonsusceptible to S. bromi in greenhouse inoculations. Preliminary data indicate that high resistance may be obtained by selection.

Stagonospora bromi A. L. Sm. & Ramsb., described as a new species on Bromus ramosus in England in 1914 (3), was first isolated from leafspots on B. inermis at University Park, Pennsylvania in 1958. In a subsequent field study of foliar diseases of B. inermis, the fungus was isolated from leafspots on both primary and secondary growth of the grass. According to Sprague (4), by 1950 S. bromi had been reported on eight Bromus species in Kentucky and six mid-western States. Sprague (5) also recently reported a Phyllosticta sp. associated with S. bromi and indicated his belief that they were stages of the same fungus.

Although S. bromi is not economically important every year, reports by Hardison (1, 2) indicate that it can be a destructive parasite of Bromus species. Except for the brief account by Tiffany (6), reports on the physiology and pathogenicity of S. bromi were not found in the literature. It seemed, therefore, that more detailed information would be valuable in meeting the threat posed by this pathogen of the bromegrasses.

SYMPTOMS

Lesions produced on B. inermis are dark brown, elongated, and often pointed at the ends, the inner portions becoming light tan as the lesion expands. Pycnidia develop within the infected tissue. Although similar in appearance to brown spot caused by Helminthosporium bromi Died., lesions are less angular in outline and may be distinguished by the light central area and the pycnidia in the infected tissue. Scald lesions (Rhynchosporium secalis (Oud.) J. J. Davis) in an early stage of development are similar in outline but the central portion appears greyish and water-soaked rather than light tan.

MORPHOLOGY

The fungus grew best and fruited abundantly on dehydrated lima bean agar. The light-grey colonies are circular, becoming dark with pycnidia as they expand. Pycnidia are dark brown to black, ostiolate, and pear-shaped (Fig. 1), and they develop on and beneath the surface of the medium. The dimensions of 50 or more pycnidia in culture averaged 140 x 187μ (73-218 x 104-296μ), whereas pycnidia that developed in artificially inoculated leaves of B. inermis averaged 72 x 94μ (52-94 x 62-135μ). Conidia are hyaline, fusiform, mostly 3-septate when mature, usually straight but occasionally curved. One or both ends may be pointed. They are discharged from the ostiole of the pycnidium as a pale-pink slimy mass. The average dimensions of 100 conidia in culture are 20.6 x 3.0μ (15.8-27.8 x 2.3-3.4μ), and from infected leaves of B. inermis (artificially inoculated) 19.0 x 3.1μ (11.3-27.8 x 2.6-3.4μ).

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[1] Plant Pathologists, Crops Research Division, Agricultural Research Service, United States Department of Agriculture. Contribution No. 194 of the United States Regional Pasture Research Laboratory, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, University Park, Pennsylvania, in cooperation with the 12 Northeastern States.

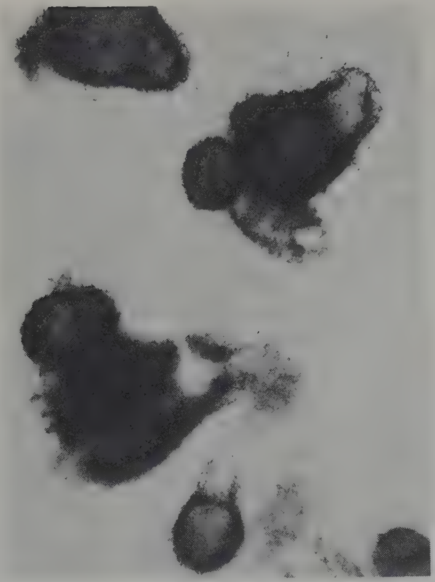

FIGURE 1. Pycnidia of Stagonospora bromi on lima bean agar. Note spore discharge. 100X

In cultural studies no Phyllosticta stage was observed to be associated with S. bromi. However, a species of Ascochyta (with a Mycosphaerella perfect stage) was isolated from a Stagonospora-type lesion.

TEMPERATURE RELATIONSHIPS

Colony Growth: Two experiments were conducted in which three isolates of S. bromi were grown on lima bean and malt-extract agar. Cultures were started by placing a 4-mm disc of mycelium in each plate. Three plates of each isolate were incubated in darkness at 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. On both media the average colony diameters of all isolates were highest at 25° and slightly less at 20° after 11 days of incubation.

Pycnidial Formation: The above cultures on lima bean agar were also examined to determine the effect of temperature on pycnidial formation and spore discharge. Well-developed pycnidia formed in 3 days at 20°, 25°, and 30° C, in 4 days at 15°, and in 11 days at 10°. No pycnidia formed at 5° and 35°.

Spore Discharge from Pycnidia: After 8 days a few pycnidia exuding pale-pink spore masses were observed in cultures incubated at 15°, 20°, and 25° C, but at the other temperatures spore discharge had not occurred. After 11 days the sporulating pycnidia in each of three microscopic fields contiguous to the 4-mm inoculum disc in each culture plate were counted. The cultures incubated at 20° had the greatest numbers of sporulating pycnidia. The numbers were lower at 25° and 30°, and least at 15°.

In another experiment with one isolate, 4-mm discs (from cultures previously incubated at 20° C) containing immature pycnidia were transferred to plates of lima bean agar and incubated at 15°, 20°, 25°, and 30°. Ten discs were incubated at each temperature and readings were made by counting the sporulating pycnidia. The average number of sporulating pycnidia per disc after 6 days was highest at 20°, and progressively less at 15°, 25°, and 30°.

Tests were also conducted to determine the effect of previous incubation temperature on spore discharge. The results of three separate experiments were variable, and no consistent effect was detected.

Germination of Conidia: Spore germination studies were conducted by the microscope-slide method using conidial suspensions of two isolates from B. inermis. Two circles were made on each slide with a glass-marking pencil. Petri-dish moist chambers containing two slides were placed in incubation chambers at 5°, 10°, 15°, 20°, 25°, and 30° C. After the slides had become adjusted to incubation temperature, one drop of conidial suspension was placed in each circle. One hundred conidia in each drop of suspension were examined after 4, 8, and 24 hours of incubation.

The percentage germination of both isolates at the three time intervals was consistently highest at 25° C. The combined averages of both isolates at the different temperatures after 8 hours of incubation were: 1% at 5°, 10% at 10°, 42% at 15°, 57% at 20°, 66% at 25°, and 45% at 30° C. The germ tubes are thread-like and may arise from any cell of the conidium.

Disease Development: Artificial inoculations were conducted by spraying plants of B. inermis in 4-inch pots with a conidial suspension of S. bromi. The plants were then covered with transparent polyethylene bags (which were removed after 3 days) and kept in incubation chambers. Check plants were sprayed with tap water.

In one experiment, seedlings of Lincoln variety in four pots were incubated under fluorescent light at 15°, 20°, 25°, and 30° C. Very numerous small brown spots were evident after 3 days on plants kept at 15°, 20°, and 25°, but not at 30°. After 5 days, individual lesions on plants kept at 20° were most numerous, 1.5-2.0 mm long, and becoming light in the centers; at 15° and 25° - 1.0-1.5 mm long, while at 30° lesions were fewer and smaller.

In two other experiments, inoculated plants of Lincoln and Saratoga varieties were incubated in chambers in diffuse daylight at 21°, 24°, and 27° C. There were four pots and one check (30 pots) of each variety at each temperature in one experiment, two pots of Saratoga, six pots of Lincoln, and three or more checks in a subsequent experiment. Although disease was slight in the first experiment, most disease developed in the range of 21° to 24° after 12 days. The plants were then cut back, fertilized with nitrogen, and re-inoculated as before. Ratings on lesion size and disease severity were made after this inoculation. Using a scale of 0 to 10, the average disease severity ratings were: 4.3 for plants kept at 24°, 3.5 at 21°, and 2.4 at 27° after 10 days of incubation. Using a scale of 1 to 5, the average ratings on lesion size were: 3 at 24°, and 2 at 21° and 27°

The results of these inoculations indicated the optimum temperature for infection and disease development to be between 20° and 24° C, probably nearer 24°. Plants of Saratoga variety appeared to be slightly more susceptible than Lincoln.

Pasture Laboratory selections of B. inermis were screened for resistance to S. bromi. Lines varied from very susceptible to resistant.

HOST RANGE

In two greenhouse experiments, seedlings in four 4-inch pots of the following grass species were inoculated with two isolates of S. bromi by spraying with conidial suspensions: Manchar smooth bromegrass (Bromus inermis), orchardgrass (Dactylis glomerata), timothy (Phleum pratense), meadow fescue (Festuca elatior), tall fescue (Festuca arundinacea), redtop (Agrostis alba), perennial ryegrass (Lolium perenne), reed canarygrass (Phalaris arundinacea), and Pennoll wheat (Triticum vulgare). One check pot of each grass was sprayed with tap water. Immediately after spraying, each pot was covered with a transparent polyethylene bag and left on a greenhouse bench at 23° to 26° C. The bags were removed 2 or 3 days later. A moderate amount of disease developed on bromegrass leaves after 16 days. No disease symptoms developed on leaves of the other grass species inoculated.

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TUPELO LESION CAUSED BY FUSARIUM SOLANI

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Abstract

A trunk lesion of swamp tupelo, first observed in Louisiana during 1959, is characterized by stem swelling and roughened bark caused by the killing of small areas of cambium in successive years in the same general stem area. Fusarium solani appears to be the causal fungus.

In October 1959, scattered trees with pronounced lesions were found in stands of swamp tupelo (Nyssa sylvatica var. biflora) near Thibodaux, Louisiana. Sectioning of infected trees showed that some lesions had formed during most years since 1940. The lesions have not been observed in other tupelo swamps.

The lesions, which individually are only a few square inches in extent, result from the killing of limited patches of cambium. The patches heal and are soon covered by callus tissue, but each year more killing and healing take place in the same general area of the bole. This localized killing causes roughened bark and swollen areas on the stem (Fig. 1). When overgrown, the patches of bark and dead wood constitute a defect which will considerably devalue lumber cut from the trees.

Isolations from the margins of the lesions yielded only one fungus. This was identified as Fusarium solani. Dr. W. C. Snyder of the University of California confirmed the identification.

Six pathogenicity tests were made at Stoneville, Mississippi, from 1959 to 1962. On December 1, 1959, a small wound was made on each of twenty 1-year-old tupelos and inoculum from fresh cultures of F. solani was placed in each of the wounds, which were then covered with moist cotton held in place with tape. Ten check trees were treated similarly, but with sterile agar instead of the fungus. Five months later cankers extending 1/2 to 2 inches beyond the limits of the wound were observed on 65% of the inoculated seedlings. An additional 25% of the seedlings had smaller cankers. All of the checks had healed without infection. F. solani was reisolated from the cankers.

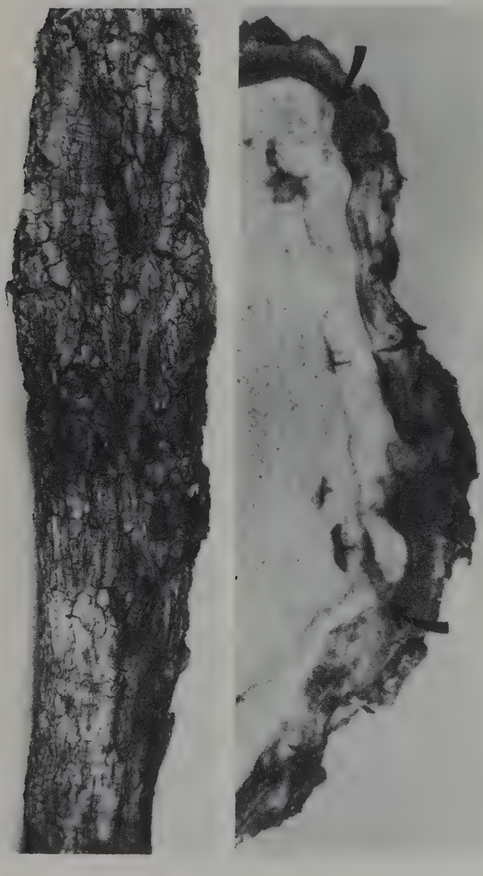

FIGURE 1. Typical lesions on swamp tupelo. Arrows in right view point to dead phloem and cambium.

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[1] Plant Pathologist, Southern Forest Experiment Station, Forest Service, United States Department of Agriculture. Assigned to the Stoneville Research Center, maintained at Stoneville, Mississippi, in cooperation with the Mississippi Agricultural Experiment Station and the Southern Hardwood Forest Research Group.

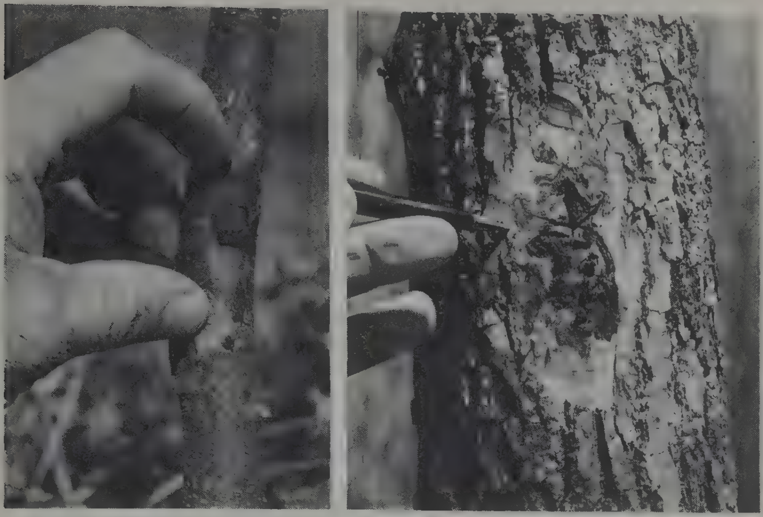

FIGURE 2. Left -- Canker on 1-year-old tupelo seedling 4 months after inoculation with F. solani. Right -- Canker on 30-year-old tupelo 18 months after inoculation.

Twenty 2-year-old trees were inoculated in April and ten in August of 1960; all healed without developing cankers. By contrast, 100-percent cankering resulted from inoculations made during the dormant season -- November 1960, January 1961, and March 22, 1962 (10 different saplings inoculated on each of these three dates).

Twenty wound inoculations were made on 30-year-old tupelo growing near McCool, Mississippi, in October 1960. Six months later 75% of these were infected. Eighteen months after inoculation some cankers had healed, but 50% were still active.

Figure 2 shows cankers that developed from the inoculations.

These tests indicate that the strain of F. solani isolated from the tupelo lesions can produce cankers, but infections occurred only from inoculations during the dormant season. The fungus probably remains viable in phloem tissue and reinfects in subsequent years.

This nonkilling disease so far does not appear to be widespread, but it is potentially important as a cause of lumber degrade.

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FOMES ROOT ROT FOUND IN FIVE THINNED, NATIVE WHITE PINE STANDS IN WEST VIRGINIA[1]

James H. Ginns and William H. Gillespie[2]

Root rot of conifers, caused by Fomes annosus (Fr.) Cke., occurs commonly in thinned plantations of various species throughout the northeastern States. During the past few years, reports of the disease in other States have increased steadily. It was first reported from West Virginia in 1958 by R. P. True (West Va. Agr. Exp. Sta.) who observed the fungus fruiting on living native red cedars (Juniperus virginiana) at one location in Greenbrier County. In November, 1959, Marvin Fowler (N.E.F.E.S.) identified the disease as the one responsible for mortality of white pine (Pinus strobus) in Clover Run Plantation on the Monongahela National Forest in Randolph County. The latter report (personal communication) and succeeding oral communications indicate that the eventual damage to this 70-acre plantation will be great.

Eastern white pine is at present the most valuable native coniferous timber tree in West Virginia. In general, the plantations in the State are not old enough for thinning, but many are approaching this age, and Christmas tree plantations are being thinned. To ascertain the present extent of the disease and the probability of future damage, thinned, native stands were examined for Fomes root rot.

During April, 1962, three areas in Pleasants, Mercer and Raleigh counties and two areas in Pocahontas County were examined initially and the Greenbrier center was re-examined. Stands were selected from areas that had been thinned from 5 to 7 years ago and were readily accessible. In most instances the thinning was an initial selection cutting, but trees had been cut in the Pleasants County area at least twice in the last 10 years and in one of the Pocahontas County areas the stand had been completely removed. In the latter area fruiting bodies were observed only on stumps. Elsewhere, examination consisted of checking for thin crowns, or yellow crowns, or both, and obviously dying or dead trees in the residual stand. This was followed by identification of fruiting bodies on nearby stumps. Although not every instance was verified by culturing, increment cores taken at the ground line from several thin-crowned living trees yielded Fomes annosus in culture.

In the Pleasants County area, six symptomless trees previously undercut and left standing were sampled below the cut. None of these samples yielded the fungus.

Re-examination of the Greenbrier area showed that the disease was killing infected cedars, as manifested by abundant fruiting bodies, two dead trees, and one living blowdown. Inspection of an adjacent thinned and pruned white pine plantation (Clark Tract) revealed F. annosus fruiting on stumps.

The significance of this report is increased by the fact that sporophores were found on stumps in every stand examined in five widely separated counties. In each native stand that had been logged and in which residual trees were left standing, a few trees were dying from the disease. This informal survey indicates that Fomes root rot is already a widespread problem in thinned natural stands in West Virginia and that it could become destructive in plantations.

WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION AND STATE DEPARTMENT OF AGRICULTURE

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[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 697.

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MOVEMENT OF THE BURROWING NEMATODE FROM A POTTED DUNCAN GRAPEFRUIT SEEDLING INTO PREVIOUSLY UNINFECTED SEEDLINGS GROWING IN ADJACENT POTS

E. B. Sledge[1]

INTRODUCTION

The spread of Radopholus similis (Cobb) Thorne, the burrowing nematode, through potted nursery plants is a problem of considerable importance to Florida growers. The pattern of infestations of R. similis in many cases indicates that the infection initially came from one or more potted plants placed in a group of previously uninfected plants and then moved, or was moved, into adjacent plants. This frequently happens where potted plants, judged to be free of R. similis on the basis of sanitary growing practices and nematode sampling, become infected when other potted plants not known to be nematode free have been adjacent to them for a period of time. It is generally assumed that microorganisms, such as fungi, can be spread from infected to uninfected pots by splashing water (1). Contamination is prevented by most research workers by spacing pots and watering so that there is minimum of splashing between pots. However, the author found no report in the literature of any demonstration to show whether plant parasitic nematodes can be moved, or move themselves, into an adjacent potted plant under simulated nursery greenhouse conditions.

Hannon (2) conducted a 2-month experiment to study the contamination of potted plants by R. similis. Thirty-five 6-inch pots of soil, each planted with a black nightshade (Solanum nigrum) plant, were placed on the ground in a rectangular pattern with rows seven pots long and five pots wide. Seven pots contained soil infected with R. similis and were arranged so they were surrounded by the maximum number of uninfected pots. Water was applied as needed, and no provision was made to prevent splashing. At the end of the experiment, Hannon found plants in eight previously uninfected pots contained from 1 to 47 R. similis. One previously infected pot contained no R. similis. He concluded the contamination was caused by splashing water.

To study the possible movement of R. similis into uninfected potted plants growing under simulated nursery greenhouse conditions, the following experiment was conducted by the author.

MATERIALS AND METHODS

On November 3, 1960, 18 Duncan grapefruit (Citrus paradisi) seedlings grown in fumigated soil were potted in fumigated soil in 6-inch clay pots and placed in two rings around an R. similis-infected seedling potted in R. similis-infected soil (Fig. 1). Pots were arranged to provide maximum contact with adjacent pots. The pots were situated on a clean, flat, asbestos bench top in the greenhouse.

The plants were maintained for 18 months under conditions similar to those in a commercial operation. Sufficient water was applied to all plants with a water-breaker by moving the stream of water over the entire group of pots. No attempt was made to influence splashing. The seedlings were fertilized at regular intervals to insure normal growth.

The experiment was terminated on May 4, 1962, 18 months after its commencement. The tops of all plants, which by this time were from 18 to 27 inches tall, were removed and each root system incubated separately for 6 days after the method of Young (3). The number of R. similis recovered from each plant was counted and recorded.

RESULTS

The total number of R. similis recovered from each plant is shown in the respective pot position in Figure 1. Counts include males, females, and larvae.

No R. similis were found in the root system of the originally infected seedling, that is, in pot A. Pot D was the only plant of the six adjacent to pot A that was positive, with 17 R. similis recovered from it. Of the six plants adjacent to pot D, only two, pot L with 52 and pot M with 165 R. similis, were positive. Two of the four plants adjacent to pot M were positive, pots D and L. The other two, pots E and N, were negative.

By the end of the experiment, the roots of all plants had grown through the bottom openings

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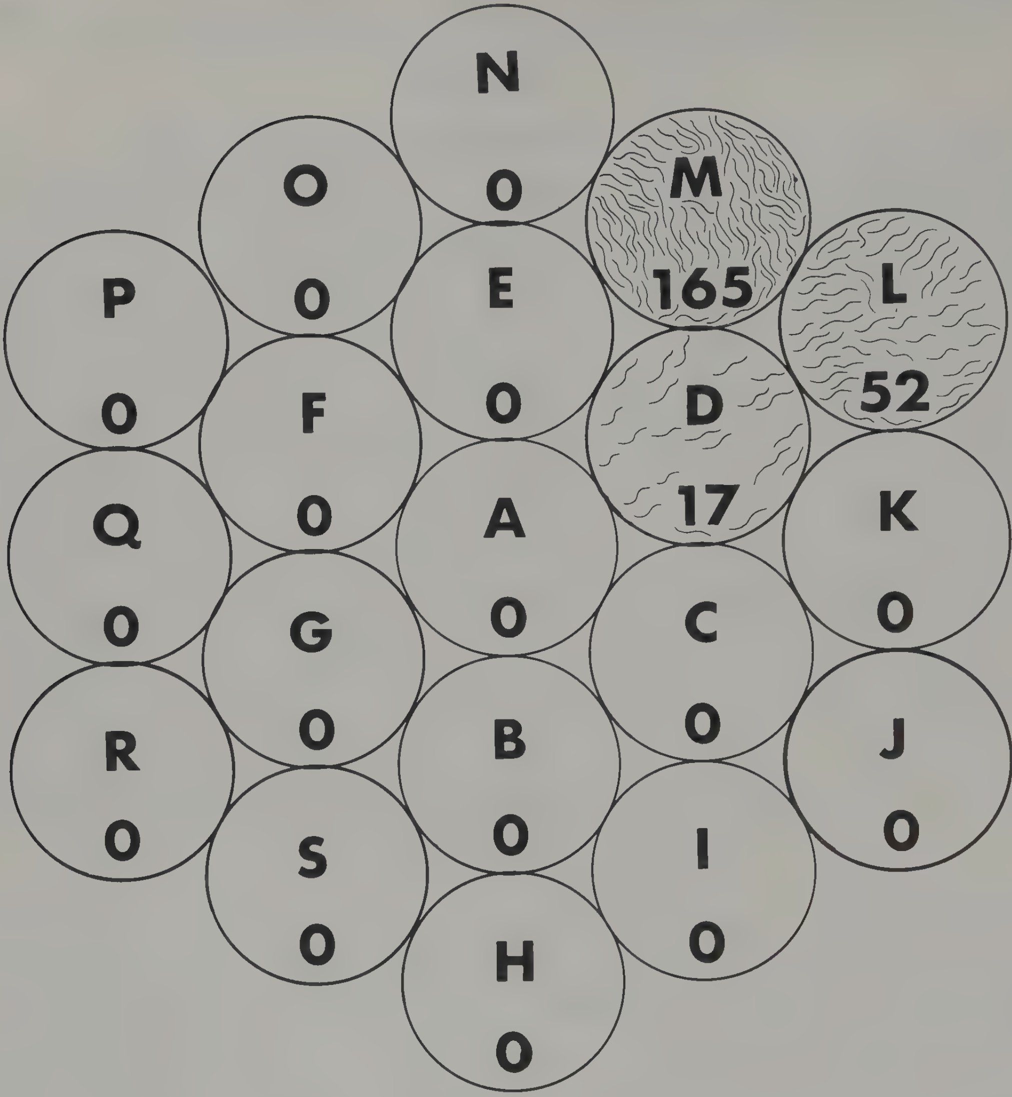

FIGURE 1. Diagram of pot arrangement with number of Radopholus similis recovered from each plant at the termination of the experiment.

of their respective pots. However, none of the roots had grown to, or beyond, the outside bottom edge of any pot. Thus, there was no root contact between any of the plants via which R. similis could have moved during the experiment.

During the experiment small amounts of soil frequently splashed from one pot to another and onto the bench top, forming thin patches of soil in the areas between the pots. With each application of water these patches changed in shape, thickness, and position. Leaves and other kinds of debris were added to this soil as the plants grew and the lower leaves fell away. The small patches of litter thus formed remained moist for several hours after the plants had been watered. However, none of these observed patches of litter were ever extensive or permanent enough to form a connection between any two pots.

Green algae grew in very thin patches along the rims and sides of most of the pots. Algal growth was not extensive and did not prevent the rims of pots from drying a few hours after watering. None of this material was examined for the presence of R. similis.

There was no observed difference in the top growth of any of the seedlings during the experiment. After approximately 12 months' growth, the tops were large enough to cast considerable shade on the pots and soil below.

DISCUSSION

The results show that *R. similis* can be moved, or move itself, from infected pots into adjacent potted plants growing under greenhouse conditions. The results do not indicate how the worms got into the pots in which they were found at the termination of the experiment. This movement could have occurred by splashing water moving the nematodes themselves or clumps of soil containing nematodes; or migration of the nematodes via (a) a moisture film on the clay pots and bench top, (b) the debris and soil accumulated on the bench top, or (c) the algal growth on the pot rims.

Because of the conditions of the experiment, however, it is likely that the worms were carried into adjacent pots by splashing water or in clumps of soil containing them.

The pattern of nematode movement appears (Fig. 1) to have occurred in the shape of the letter L, from pot A to pots D, L, and M. But to assume the nematodes reached only the pots in which they were ultimately found is unsound, since they were initially in pot A but not there at the end of the experiment. The absence of *R. similis* from pot A is not understood, but it is consistent with my general observations on population fluctuations of this parasite on potted Duncan grapefruit seedlings. Populations seem to undergo cycles of high and low densities for approximately a year. This phenomenon is apparently related to host and environmental conditions. Populations then frequently continue to decline to the point where seedlings may harbor a few or, in some cases, no *R. similis*. Thus, in this experiment possibly other plants in addition to pots D, L, and M were invaded, but *R. similis* populations reached a peak and died out before the end of the experiment.

Acknowledgment: Appreciation is expressed to Dr. C. I. Hannon, Assistant Nematologist, Citrus Experiment Station, Lake Alfred, Florida, for the information concerning his experiment.

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THE NATURAL OCCURRENCE OF FOMES ANNOSUS AND ASSOCIATED FUNGI IN SLASH PINE STUMPS[1]

J. S. Astin, Jr. and Chas. H. Driver[2]

Summary

The occurrence of Fomes annosus (Fr.) Cke. and associated fungi was studied in slash pine stumps 6 to 9 months after cutting in south Georgia during the summer of 1961. Although trees were cut during a period of high infection potential, only 10% of the stumps yielded F. annosus. The presence of F. annosus was shown more reliably by tissue isolation than by other methods. This fungus was recovered more often from the lateral roots than from the stump itself. Peniophora sp., various wood staining fungi, Trichoderma spp. and other imperfect fungi also were present in the stumps.

INTRODUCTION

The suggestion by Campbell and Hepting (1) in 1954 that the root rot fungus Fomes annosus might someday become a factor in the management of slash pine in the South is being substantiated by recent reports. Surveys during 1960 by Driver and Dell (2) and Powers and Boyce (5) showed the presence of the disease in thinned slash pine plantations of South Carolina, Georgia, Florida, Alabama, and Mississippi. These reports indicate that the disease is widespread in thinned slash pine plantations in the South.

Reports from Great Britain show that the disease is becoming widely established in forests and is a serious threat to future timber production (9). Rishbeth (7) reports the use of toxic chemicals and preservatives to protect stumps in an attempt to prevent air-borne infection by F. annosus in British forests.

Because of Rishbeth's success with stump treatments, a test to evaluate stump protectants under natural conditions of infection in slash pine plantations in the United States is currently being conducted. This study of untreated control stumps was made to determine the fungi present in them resulting from natural infection.

MATERIALS AND METHODS

The study was made in a 13-year-old previously unthinned slash pine plantation at the Southlands Experiment Forest located in the middle coastal plain in Decatur County, Georgia. This stand surrounds a 1.7 acre, 24-year-old slash pine plantation with extensive annosus root rot damage described by Driver and Dell (3). All trees in this area were cut on December 15, 1960. Average daytime temperatures were 60° to 70°F for 1 week before and 1 week after the cut. Relative humidity averaged above 80%. Spore trappings by the Rishbeth pine disk method (8) with an exposure period of less than 5 minutes demonstrated that spores of F. annosus were widely distributed throughout the area during cutting operations.

Forty stumps were sampled during June, July, and August of 1961, 6 to 9 months after felling the trees. Transverse and longitudinal stump sections and lateral root sections were analyzed. Stump heights ranged between 4 and 9 1/2 inches with an inside bark top diameter of 4.5 to 9.5 inches. A disk 5 to 10 cm thick was hand sawn from the top of each stump. Then a longitudinal section about 10 cm thick was split off the side of the stump. This section passed through at least one of the four lateral roots of each stump sampled and it extended below the soil surface to include the root origin area. A root section about 6 inches long was removed from a lateral root in each of the four cardinal directions. A second disk was cut partially through and left attached to the stump which was then covered with kraft paper and sealed at the base with soil and left for field incubation. All cutting tools were sterilized with ethanol. Samples were wrapped in paper and brought immediately to the laboratory where isolations were made.

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[1] This research was supported by a grant from the Georgia Forest Research Council and International Paper Co. Journal Series Paper No. 251, College Experiment Station.

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Three methods were employed to detect F. annosus. The primary method consisted of culturing random bits of wood from characteristic decay areas. All sections were split again in the laboratory and tissue samples removed aseptically and placed on 2% malt extract agar slants. Cultures were incubated at room temperature, identified and grouped in five classes as soon as diagnostic characteristics developed.

Secondly, the same stump and root samples after culturing were wrapped in moist paper, stored in perforated cans at room temperature and examined 2 to 3 weeks later for the appearance of F. annosus conidiophores on the freshly exposed wood surfaces (6).

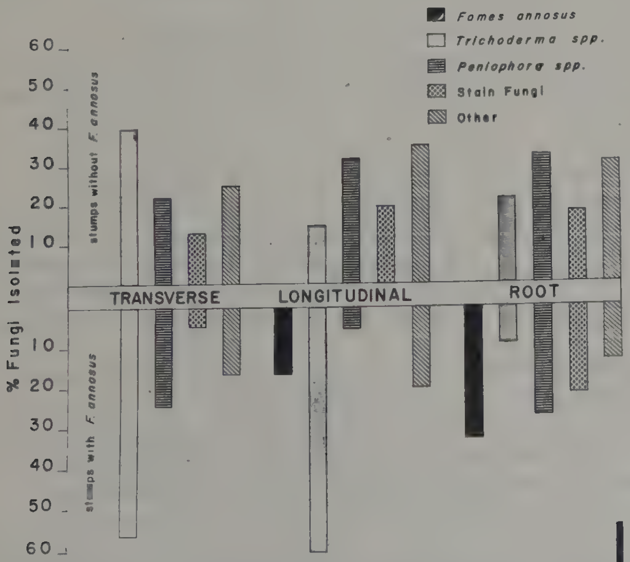
The final method was accomplished by the examination of stumps previously prepared for field incubation. This consisted of the removal, after 2 to 3 weeks, of the second disk and the examination of the interface between the stump and this disk for the development of conidiophores of F. annosus.

When F. annosus was found in a stump, additional isolations were made to determine the nature and extent of fungal associations. Three more longitudinal sections were taken and the root yielding F. annosus was sampled over its entire length to determine the direction and extent of its colonization.

RESULTS

Fungi were grouped to indicate the nature of their associations in the stumps. Peniophora sp. was equally frequent in both the stump body and the roots. Stain fungi were more frequent in the roots. Stain fungi including Leptographium and Chalara species were common colonizers occurring in about half of the stumps. Twenty to 30% of the stumps or their roots yielded Graphium and Ceratocystis. Ninety-five percent of the stump root combinations contained Trichoderma spp., while 97% contained other fungi. The latter included the following: Fusarium spp. (50% of the stumps); Paecilomyces sp., Diplodia sp., Cephalosporium sp., Cytosporella sp., and Gliocladium sp. (10 to 20% of the stumps); Botrytis sp., Geotrichum sp., Chalaroposis sp., and Phoma sp. (3 to 10% of the stumps).

There seemed to be a relationship between the resin coverage of stump surfaces and the development of decay observed in stumps 6 to 9 months after cutting. In stumps having moderate to heavy resin deposits completely covering the surface, a sound, generally undecayed condition was observed. Some were alive and contained apparently active cambial tissue. Roots of these stumps also were usually healthy, indicating possible grafts with roots of adjacent liv-



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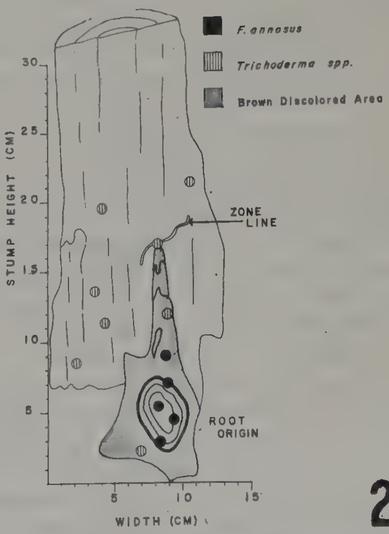

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FIGURE 1. Distribution of five groups of fungi isolated from the transverse and longitudinal sections of stump bodies and root sections of 36 slash pine stumps not infected with F. annosus and four stumps infected with F. annosus.

FIGURE 2. Location of isolates removed 9 months after felling from the longitudinal section of a decaying slash pine stump infected with F. annosus.

Table 1. Distribution of five groups of fungi in 40 slash pine stumps 6 to 9 months after felling.

Stump part	% stumps yielding isolates of				
	F. annosus	Trichoderma spp.	Peniophora sp.	Stain fungi	Other
Stump	3	75	60	50	83
Roots	10	70	60	65	90
Either	10	95	70	75	97

Table 2. Extent of colonization of *F. annosus* and nature of fungus associates in lateral roots of four naturally infected slash pine stumps.

Root no.	Species isolated from root origin area in stump	Distance from stump F. annosus isolated (in inches)	Principal species in roots
1	*Trichoderma* sp.	3	*Peniophora* sp. *Trichoderma* spp.
2	*F. annosus*/*Trichoderma* spp.	42	*F. annosus*
3	*F. annosus*/*Trichoderma* spp.	3	*Chalara* sp., *Leptographium* sp. (stain fungi)
4	*F. annosus*/*Trichoderma* spp.	23	*F. annosus*

ing trees. Stain fungi, various Fusaria as well as other imperfect fungi, bacteria and uninfected bits of tissue were recovered from these "sound" stumps.

Data for the 36 stumps not colonized by *Fomes annosus* are illustrated in Figure 1. *Peniophora* sp. was recovered to a greater extent from longitudinal sections and roots than from transverse sections. The occurrence of stain fungi differed little as to position in stumps. *Trichoderma* spp. were more frequent in the upper stump than in the lower stump and roots, while all other fungi were found to a greater extent in the lower stump and roots.

Kinds and relative frequency of fungi recovered from the four stumps infected with *F. annosus* are also shown in Figure 1. Surprisingly, *F. annosus* was not isolated from the top of stumps. The principal organism isolated from this area was *Trichoderma* spp. Stain fungi were rarely obtained from the transverse sections. Longitudinal sections yielded all groups presented in Table 1 except stain fungi.

Peniophora sp. was found extensively in the roots of two stumps but not in those of the other stumps and it was never found in the same root with *F. annosus*. Stain fungi, such as *Leptographium* sp. and *Chalara* sp., were found to a limited extent in the root systems of two stumps. When *F. annosus* occurred, it was usually the only fungus recovered from the proximal portion of individual lateral roots. However, *F. annosus* was present in association with *Gliocladium* sp., *Leptographium* sp. and *Fusarium* sp. as well as *Trichoderma* spp. in the same 6-inch root section.

Additional isolations were made from roots containing *F. annosus* to determine the extent of colonization distal to the stump. This information is presented in Table 2.

During laboratory incubation of wood sections that had previously yielded *F. annosus* by culturing, the presence of the organism was confirmed by the development of typical conidiophores on samples from three of four stumps. Conidiophores of *F. annosus* developed on 10 of 29 pieces. An additional 11 pieces were covered with a white mat of mycelium believed to be *F. annosus* but it failed to develop the typical conidiophores and thus could not be confirmed as *F. annosus*. Field incubation confirmed the presence of *F. annosus* in only one of the stumps.

Fomes annosus was isolated originally from the roots of three stumps and the roots and body of a fourth stump. In a more extensive analysis of the four stumps, it was found to a limited degree in the upper portion of each of them.

DISCUSSION

This study showed a relatively low effective colonization of slash pine stumps by *F. annosus*. It is surprising that only 10% of the stumps contained *F. annosus* in view of the fact that spore trapping at the time of cutting showed a high incidence of inoculum in the air. *F. annosus* occurred primarily in the roots and root origin areas of these stumps and in no case was it found closer than 19 cm from the top of the stump. The possibility of initial invasion of *F. annosus* other than through stump surfaces cannot be eliminated. Since some of the stumps were in an advanced condition of decay, any attempt to study the early stages of decay stumps should be analyzed prior to 6 months after felling. The results indicate that the most accurate way to determine the presence of *F. annosus* in this test was by tissue isolation.

The data in Table 2 were obtained after a 6-inch root section had been removed 2 to 8 weeks prior to culturing. *Trichoderma* spp. could have entered during this interval. *Trichoderma* spp. were associated with *F. annosus* in root origin parts of the stump but not in the roots, suggesting that it probably did not enter the exposed roots.

Meredith (4), working with Scots pine in British plantations, reported that 12 months after felling the distribution of fungi over the surface of incubated transverse stump sections provided a fair guide to their distribution in lateral roots. In this study, however, transverse sections taken from tops of the 6- to 9-month-old stumps did not bear this out for the slash pine species.

Rishbeth (6, 7) has described the replacement of *F. annosus* by *Trichoderma viride* and Meredith made similar observations in stumps more than 2 years old. In the present study, *F. annosus* was often isolated in close proximity to tissue yielding *Trichoderma* spp. (Fig. 2). On several occasions, *F. annosus* and *Trichoderma* spp. were observed fruiting on the same piece of tissue. British work indicates that imperfect fungi are infrequent in stumps less than 1 year old (4). In contrast, we found them to be relatively abundant 6 to 9 months after cutting.

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A METHOD FOR ASSAYING NEMATOCIDAL ACTIVITY OF EXPERIMENTAL CHEMICALS

D. E. Johnson and Bert Lear[1]

Summary

Mixing root-knot nematode-infested soil and experimental chemicals in polyethylene bags has proved to be a safe and efficient method of dispersing candidate materials throughout a soil mass. After a storage period the nematocidal activity of a chemical is evaluated by growing a susceptible plant in replicate lots of treated soil.

Many methods for determining the nematocidal activity of experimental chemicals have been described (Christie (1), McBeth and Bergeson (2), Tarjan and Cheo (3), Taylor, et al. (4)) and are currently used by various workers. Two methods are used for screening chemicals prior to field testing: 1) The "water screen" involves exposing nematodes, free-living or plant-parasitic, to various concentrations of solutions or emulsions of candidate chemicals for various time intervals. At the end of the exposure, the nematodes are examined and the toxicity of the chemical is evaluated on the basis of nematode movement. 2) The soil test entails adding the chemical directly to a soil already infested with nematodes. The subsequent storage period may take place in either an open or closed container. After an appropriate interval, the soil is removed from the container and planted with a host for the test nematode. Nematode control by the toxicant is evaluated by recovering the nematodes from the soil or plant and counting directly or, in the case of root-knot nematodes, by evaluating the degree of root galling.

The method used by the Department of Nematology at the University of California, Davis for screening experimental chemicals is a modification of the second type. Adoption as a standard method by several companies indicates interest in this method.

TEST DESCRIPTION

The soil used in this screening procedure is of a fine sandy loam texture with a moisture equivalent of 12.3%. A sufficient quantity of this soil infested with Meloidogyne javanica javanica Chitwood is screened through a 2-mm sieve to remove roots and extraneous materials. The root-knot nematode population was derived from a single egg mass and is maintained in the greenhouse as an inoculum source.

The screened soil is next mixed in a concrete mixer for 15 or more minutes to provide the required aliquot uniformity. In the event the mixed soil contains too little moisture, water is added during the mixing until the soil moisture reaches a value of about 3/4 moisture equivalent.

One pint of soil (about 375 grams) is placed in a polyethylene bag (3 x 6 x 12 inches) that is closed to maintain moisture until treatment.

A solution, when possible, of each experimental chemical is added at the following rates to the requisite number of bags, usually three bags per rate:

Rate 1 - 0.005 ml or g of actual material.
Rate 2 - 0.01 ml or g of actual material.
Rate 3 - 0.02 ml or g of actual material.

In addition to the experimental chemicals, a standard nematocide such as 1,3-dichloropropene, 1,2-dichloropropane mixture (D-D Soil Fumigant), at the same rates as experimental chemicals, and an untreated check are included in each series.

After the chemical is added to the soil, the soil and chemical are mixed by shaking and kneading soil in the polyethylene bags. The bag of treated soil is then placed in a 1-pint widemouth mason jar, sealed, and the jars stored for 7 days at 75° F.

After the 7-day storage the soil is transferred from the bag to clean 4-inch clay pots on a greenhouse bench and the soil kept moist for an additional 7 days. Next, a single tomato seedling is transplanted into the pot to grow for 4 to 6 weeks, after which time the soil is washed from the tomato roots and the galls on the roots counted.

If a cyst-forming nematode (Heterodera sp.) is substituted for M. javanica, the entire soil mass as well as the roots are washed onto a 60-mesh screen to recover the cysts. Control is evaluated on the basis of the number of white females present.

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Table 1.

Treatment	Dose/pint (ml or g)	Galls per plant 1	2	3	Mean
Check		57	111	94	87
D-D	0.005 ml	57	57	90	68
	0.01	0	3	4	2
	0.02	0	0	0	0
Exp. Chem. #1	0.005 g	66	69	68	68
	0.01	43	66	55	55
	0.02	67	84	49	67
Exp. Chem. #2	0.005 g	1	1	14	5
	0.01	0	0	0	0
	0.02	0	0	0	0

Polyethylene bags are discarded after a single use to prevent cross contamination of succeeding samples.

Experimental chemicals are formulated by a dilution of 1 to 10 weight-volume for solids or 1 to 10 volume-volume for liquids.

Chemicals that control root-knot nematode in this test are subsequently tested in open-top containers as well as against other nematodes, while those that fail the test are discarded.

This system of chemical screening has been satisfactory for several years. The combination of closed system and adequate mixing of soil and toxicant tests the intrinsic nematoxicity of candidate chemicals without regard to vapor pressure considerations. Further, the chemical tested is subjected to any inhibitory effects that might be inherent in soil. Also, the nematodes are treated under conditions similar to those that might be experienced under field conditions.

The inexpensive polyethylene bags used in this testing method perform a dual function. The bags provide a rapid and safe method of mixing soil and toxicant and they also eliminate the necessity of washing the jars after each use. Also, jar lids themselves last longer because soil, which damages lid gaskets, does not lodge on the lips of the jar.

Representative data from a single test are shown in Table 1.

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STEMPHYLIUM LEAF SPOT OF COTTON REPORTED FROM LOUISIANA

J. B. Sinclair and M. N. Shatla[1]

Leaves, with lesions from cotton plants reported to be defoliating prematurely, were collected in Bossier, Concordia and West Carroll parishes. In general, leaf spotting consisted of three patterns. There were leaf spots found with light centers and red margins typical of those produced by *Cercospora gossypina* Cke. The second type of leaf spotting showed large, brown, spreading lesions with concentric rings and tended to be concentrated near the leaf margins. The third type of lesions were smaller and more circular than those above, and showed concentric rings. However, these spots were found scattered over the leaf surface.

In West Carroll Parish cotton plants, within about 1/3 of an acre in a single field, were found to be severely affected by the latter two leaf spots. The area of unhealthy plants was well defined, consisting of four outside rows the length of the field and plants along the turn row. All the leaves on affected plants had lesions, even young leaves at the top of the plants. The plants collected in Concordia Parish had symptoms similar to those collected in West Carroll Parish, but leaf spotting was not as severe. The affected plants were restricted to a high ridge along the edge of a single field. Leaves with similar spots were collected in Bossier Parish, but these were found scattered in a single field and only on old leaves near the bottom of the plants.

Specimens were sent in or collected by the authors from these locations and incubated under moist conditions to induce spore production. Microscopic examinations were made.

Spores of *Alternaria* sp., probably *A. tenuis* Auct.[2], were found associated with the large, marginal, spreading leaf lesions with concentric rings from all three locations. Spores of *Stemphylium* sp., probably *S. botryosum* Wallr.[2], were found associated with the small, scattered circular lesions with concentric rings. *Stemphylium* sp. was found on leaves from both West Carroll and Bossier parishes, but not Concordia Parish.

Stemphylium and Alternaria leaf spots of cotton are reported to occur throughout the cotton belt in the United States[2]. However, a check through the Louisiana plant disease record file in the Louisiana State University Department of Plant Pathology failed to show that Stemphylium leaf spot had ever been recorded in Louisiana. Alternaria leaf spot had been reported. This report adds Stemphylium leaf spot to the records of cotton diseases found in Louisiana.

Both the Alternaria and Stemphylium leaf spot organisms are considered weak parasites or saprophytes becoming established on leaves of plants under stress from mineral deficiencies or following after other foliage diseases such as bacterial leaf spot, incited by *Xanthomonas malvacearum* (E. F. Sm.) Dows.[2] It is suggested that these severely affected plants have been suffering from drought, or other stresses, or both, thus making the plants more susceptible to both the Stemphylium and Alternaria leaf spot organisms.

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[2] United States Department of Agriculture. 1960. Index of Plant Diseases in the United States. Agriculture Handbook No. 165: 298-301.

EFFECTS OF PAPER AND SAWDUST SOIL AMENDMENTS ON MEADOW NEMATODES AND SUBSEQUENT VERTICILLIUM WILT OF TOMATOES

P. M. Miller and L. V. Edgington

Summary

Soil amendments of paper or white pine sawdust reduced populations of Pratylenchus penetrans in naturally infested soil for at least 154 days. Control of the nematodes reduced the severity of the wilt by a microsclerotial isolate of Verticillium albo-atrum. Soil amendments had no effect on the fungus itself.

INTRODUCTION

In two different experiments in 1961, the number of meadow nematodes, Pratylenchus penetrans Cobb, in both soil and roots was much lower than expected. In both experiments plants had been grown in paper cups. Previous tests conducted in plastic pots had shown no discrepancy in expected P. penetrans populations. Paper cups appeared to be responsible for the low meadow nematode populations, therefore an experiment was set up to determine whether paper would control meadow nematodes.

Meadow nematodes have been found to increase severity of Verticillium wilt. McKeen and Mountain (5) found that P. penetrans increased severity of Verticillium wilt of eggplant. Abu Gharbich, Varney and Jenkins (1) and Rich and Miller (9) found this true also for strawberries. We wanted to see if paper or paper cups might influence severity of Verticillium wilt of tomatoes.

MATERIALS AND METHODS

Six hundred grams of a fine sandy loam naturally infested with P. penetrans were used for each 4-inch polyethylene pot or 16-ounce waxed paper cup (model 1716-S of the Lily-Tulip Cup Corporation). In some of the plastic pots soil was thoroughly mixed with 2 g of either chopped paper cups or white pine sawdust. Containers were embedded in a constant water level greenhouse bench and nutrients were given as necessary. Paper amendments caused a stunting of plants. Sawdust also appeared to need more nutrient solution to prevent deficiency symptoms. Fourteen days later 50,000 microsclerotia of Verticillium albo-atrum Reinke & Berth. were added to some of the pots. Other pots received no microsclerotia. All treatments were replicated five times. At this time active meadow nematode populations in the soil were assayed by the sugar flotation-contrifugation method (6). Ten days later one 3-week-old Bonny Best tomato seedling was transplanted into each container.

Wilt symptoms appeared in 42 days and were rated 49 days after transplanting on a scale from 0 = no disease to 4 = severe disease. Another disease rating was made 10 days later. Eighty-six days after transplanting plants were harvested, weighed and the vascular browning at the first internode was observed. The roots were washed free of soil, weighed, and rated for root vigor and discoloration. The P. penetrans populations in the roots were determined by the Ferris and Bernard method (2). The amount of infestation by Rhizoctonia solani was determined by counting the number of mycelial fragments on 5 cm of roots.

To determine possible direct effects of the paper or sawdust on the V. albo-atrum, the amount of this fungus in the soil was estimated 67 and 86 days after transplanting by the Nadakavukaren and Horner method (8). Dilutions of soil 1 to 10,000 and 1 to 1,000,000 were made in agar containing 0.5% ethanol and 100 ppm streptomycin. The number of colonies of V. albo-atrum and other fungi as well were counted.

A second experiment was set up in the nematode-infested soil to which paper or sawdust had been added 73 days previously in order to test the longevity of the effect of the paper and sawdust. For this experiment another microsclerotial of V. albo-atrum was used, and 14 days later Bonny Best tomato seedlings were transplanted into containers.

Verticillium wilt ratings were made 87 days later. Root vigor, root weights, and meadow nematode populations in the roots were determined at this time.

RESULTS

Two weeks after the addition of either chopped paper or sawdust to the soil, populations of

Table 1. Effect of paper and sawdust soil amendments on populations of Pratylenchus penetrans, Verticillium wilt of tomatoes, and root condition.

Treatment	No. of P. penetrans in 30 g of soil at transplanting time	No. of P. penetrans per g of roots at harvest	Root discoloration index[a]	Verticillium wilt index at harvest[b]	Root weight at harvest
First Experiment					
Untreated soil	15	13	3.0	2.6	8.7
Paper added	3	2	1.6	1.4	8.3
Sawdust added	3	1	0.8	0.4	12.1
Second Experiment					
Untreated soil	--	13	2.1	2.0	8.2
Paper added	--	1	1.1	0.4	4.0
Paper cup	--	2	1.4	0.3	4.5
Sawdust added	--	2	1.2	0.2	10.2

[a] 0 = none to 4 very severe discoloration.
[b] 0 = none to 4 very severe wilt.

meadow nematodes were decreased to about one-fifth that of unamended soil (Table 1). The reduction in number of meadow nematodes was still evident almost 3 months later when the plants were harvested and the root infestation by the nematodes assayed. The reduction occurred regardless of whether V. albo-atrum had or had not been added to soil. Both soil amendments reduced root discoloration ratings with very obvious reduction in brownish lesions on roots. However, while the paper improved the roots' appearance it also caused a reduction in the amount of roots, whereas sawdust greatly increased the weight of roots.

The Verticillium wilt symptoms were not apparent until 42 days after transplanting seedlings into the soil, but developed rapidly thereafter in unamended soil. The wilt was absent unless V. albo-atrum had been added to the soil. The plants in soil amended with paper were not so severely diseased as those in untreated soil and those in soil amended with sawdust were almost free from disease symptoms. Vascular browning was present in all five plants in untreated soil, in two of five plants in paper amended soil and in none of five plants in soil amended with sawdust.

The second experiment, using a different isolate of V. albo-atrum, verified the results obtained earlier and extended the length of the effect of the soil amendment to 154 days with virtually identical reductions in meadow nematode populations in roots and Verticillium wilt of plants as that observed at 86 days.

The soil dilution platings 67 days after amendment revealed that a large increase in fungal population resulted from addition of chopped paper (10 to 20 fold). Colonies of V. albo-atrum were identifiable but were very few in number. A second sampling at 86 days using 80 ml of a 10^4 dilution in four plates per each of five replicates totalled 3 Verticillium colonies for untreated soil, 3 for paper amended soil and 14 for sawdust amended soil. The total number of fungal colonies for untreated soil was 9400; 12,400 for paper amended soil; and 8900 for sawdust amended soil. Both soil amendments also caused a 40% increase in root infestation by Rhizoctonia solani as shown by counts of mycelial fragments on 5 cm of roots: sawdust - 52, paper - 50, and untreated - 36.

DISCUSSION

Soil amendments, such as sawdust or paper (2 g per 600 g of soil), will drastically cut populations of meadow nematodes within 24 days and maintain a very low level for 154 days, as indicated by infested tomato roots. Verticillium wilt, known to be aided by this nematode, was reduced in severity by the soil amendments too. There are several important considerations in this unique method of controlling Verticillium wilt. First, the disease did not appear for a considerable time after transplanting tomato seedlings into the infested soil. This slow development indicates that the effective fungal inoculum potential was relatively low and typical of field conditions. The importance of meadow nematodes in predisposing plants to Verticillium may be true only when the inoculum is low. Secondly, the paper caused some undesirable reduction in plant growth. Sawdust also induced some nutritional deficiencies, probably magnesium, although this was quite easily counteracted with nutrient solutions.

These amendments, while reducing wilt, did not reduce the Verticillium population of soil. Consequently, the planting of tomato plants in soil as a technique for estimating the Verticillium population of the soil would give erroneous results, depending upon the nematode population of the soil.

The two amendments appear to act by different mechanisms in the soil. The chopped paper stimulated the total amount of fungi and also the number of saprophagous nematodes. As Mankau (3, 4) and others have shown, P. penetrans apparently fails to survive in an environment conducive to multiplication of microphagous or saprophagous nematodes. He did not observe the effect of cellulosic substances or paper cup containers which we have, but our cups were kept moist in a constant water level greenhouse bench while his cups were set upon a shelf.

Sawdust appeared to limit survival of all types of nematodes. Wilhelm, et al. (10) reported that a wood chip mulch controlled a dieback of olives associated with Verticillium. The wood chips may have reduced nematodes, as in our experiments, and consequently alleviated the dieback caused by Verticillium.

Wherever large numbers of P. penetrans were found, the roots were stunted and discolored with many small brown linear lesions, typical of meadow nematode injury. As mycelial counts on roots indicated no fungicidal effects of either paper or sawdust on R. solani, the better roots obtained by amendments with these cellulosic materials were due to preventing injury by P. penetrans.

Although McKeen and Mountain (5) reported an increase in P. penetrans numbers if the inoculum potential of V. albo-atrum was low, there was no such increase in this test. Differences in host plants, level of inoculum potential or other test conditions may account for the difference in results.

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FUSARIUM WILT OF SAFFLOWER[1]

John M. Klisiewicz and Byron R. Houston[2]

A Fusarium wilt of safflower, Carthamus tinctorius, was observed in California during May 1962. A survey in the Sacramento Valley revealed the disease in several fields of commercially grown safflower[3]. Disease incidence was estimated at 10 to 20% in most fields and as high as 50% in others. Species of Fusarium have been reported in the literature as the causal agents of or as being associated with root rot (1, 2), root-pruning diseases (4), and seed rot (3) of safflower. These and other reports in the literature on safflower diseases, however, offer no evidence suggesting a vascular wilt disease of safflower caused by a species of Fusarium.

The disease affects plants at different ages inasmuch as plants in the field were killed at early and later stages of growth. A characteristic symptom on diseased plants is a unilateral yellowing of the foliage followed by wilting (Figs. 1 and 2). Yellowing begins on the lower leaves and progresses upward on one side of the plant. The affected leaf tissue gradually turns brown and dies, and as the disease progresses the entire plant is killed. However, on older plants the lateral branches on one side may be killed, while the remainder of the plant apparently remains free from disease. A brown discoloration of the vascular bundles appears on one side of the root and stem of plants with unilateral top symptoms and becomes widespread with advanced development of the disease.

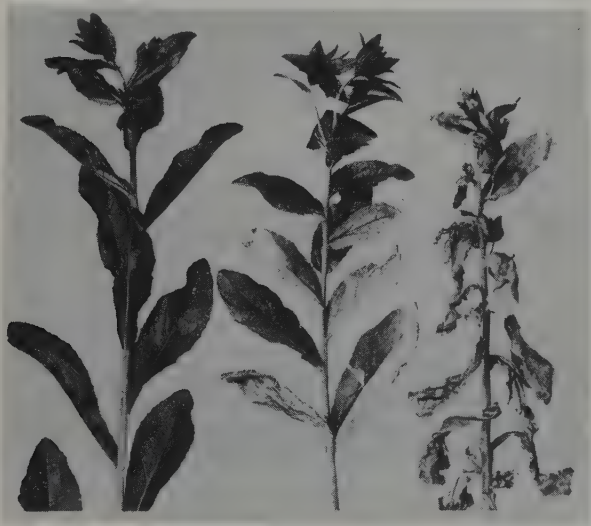

FIGURE 1. Typical wilt symptoms on safflower plant unilaterally affected (center) and plant dead from wilt (right). Healthy plant (left).

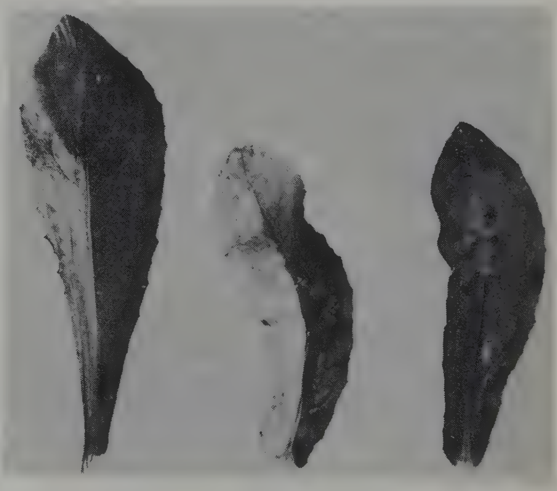

FIGURE 2. Characteristic leaf symptoms of Fusarium wilt on safflower. Healthy leaf at right.

Fusarium wilt of safflower is incited by a form of F. oxysporum Schlecht. The fungus was readily isolated from tissue of roots, stems, and leaves from diseased plants collected in the field. Pathogenicity was demonstrated on safflower by inoculating plants having injured and uninjured roots. Roots were injured by removing plants and soil intact from pots and replanting them after pouring a spore suspension over the exposed roots. Inoculations without injury to roots were made by planting seed in soil artificially infested with spores. The inoculum con-

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[3] Help in field surveys of R. C. Stanton and G. Meckfessel, Pacific Oilseeds, Inc., Woodland, California, is acknowledged.

Table 1. Susceptibility of safflower varieties to Fusarium wilt.

Variety tested	Plants with uninjured roots		Plants with injured roots	
	no. inoculated	% killed[a]	no. inoculated[b]	% killed
Pacific 1	38	100	59	100
Pacific 7	40	55	39	90
Pacific 2	42	76	38	94
US 10	43	84	44	91
Nebraska 10	39	99	35	100
Gila	42	81	45	96

[a] Surviving plants in all cases showed moderate to severe symptoms at time of final reading 4 weeks after inoculation.

[b] Total plants inoculated after 4 and 18 days of growth.

tained approximately 100, 000 spores per cc, of which 50% were estimated to be mature macroconidia. A pure, single-spore culture of the fungus was submitted to W. C. Snyder[4], who confirmed the species identification on the basis of sporulation in culture. A name will be designated for the forma specialis following additional pathogenicity tests on hosts related to safflower.

Commercial safflower varieties affected in the field were Pacific 1, Gila, and US 10. Safflower varieties Pacific 7, Pacific 2, Nebraska 10, US 10, Pacific 1, and Gila were all susceptible to the disease in preliminary greenhouse tests regardless of the method of inoculation (Table 1).

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[4] The assistance of Dr. William C. Snyder with species identification is gratefully acknowledged.

MEXICAN PALM TREES APPARENTLY KILLED BY PENICILLIUM VERMOESENI

T. R. Carpenter, W. D. Wilbur, and Wendell Young[1]

The Mexican palm, Washingtonia robusta, has been considered highly resistant to infection by Penicillium vermoeseni Biourge, whereas the closely related Washingtonia filifera is quite susceptible (1, 2, 3). No report of death of the Mexican palm caused by P. vermoeseni could be found in the literature. In April 1961, two distressed and dying Mexican palms (W. robusta) approximately 35 feet high, in a yard in Redlands, California were determined infected by P. vermoeseni. Virtually pure cultures of the organism were obtained from affected frond bases and internal trunk tissue. The fronds of one tree were wilted and drooping; the terminal bud was in an advanced stage of decay, and there was a large visibly affected area involving the entire diameter of the trunk and extending from the bud to several feet below it. Spore masses characteristic of the fungus were evident on frond bases and in the affected area. Within the other tree a large visible area of affected tissue approximately 1 foot below the bud involved the entire diameter of the trunk and extended downward for approximately 2 1/2 feet. Spore masses of the fungus were abundant on frond bases and within the affected area. The bud apparently was healthy; however, cultures of the fungus were obtained within 6 inches of it and from internal trunk tissue 6 feet below the visibly affected area. Examinations, including attempted cultures, of other aboveground tree parts and roots failed to reveal other pathogens capable of causing the observed condition of these trees. Other palms of this and other more susceptible species in the immediate vicinity apparently were not affected.

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TUBERCULINA MAXIMA ON WHITE PINE IN MICHIGAN

William J. Young

Tuberculina maxima Rostr. has been collected from a white pine canker in Charlevoix County, Michigan.

The collection was made by Mr. Leiton Nelson of the U. S. Forest Service. This is believed to be the first report of this fungus from Michigan.

MICHIGAN STATE DEPARTMENT OF AGRICULTURE, LANSING, MICHIGAN

CORRECTIONS

REPORTER, July issue (Volume 46, Number 7), page 509. In line 7 under Summary, change 3,4,5, and 6 pounds to read: 3, 4. 5, and 6 pounds.

REPORTER, August issue (Volume 46, Number 8), page 594. In Table 1, the third active compound entry should read: Cycloheximide semicarbazone, instead of Cycloheximide carbazone.

REPORTER, August issue (Volume 46, Number 8), page 610. Under Introduction, in the last line of the second paragraph, change O-2-parazinyl to read: O-2-pyrazinyl.

THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 46 Number 11

November 15, 1962

Growth Through Agricultural Progress

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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ACCEPTANCE OF MANUSCRIPTS

The volume of pertinent material submitted for publication in the Plant Disease Reporter has made it necessary to limit the length of articles accepted to twelve double-spaced typed pages, including tables, graphs, and photographs. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases.

Paul R. Miller

SUGGESTIONS FOR PREPARATION OF MANUSCRIPTS

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

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(6) PESTICIDES should be listed by their approved common names when common names are available. If the pesticide does not have an approved common name, the chemical name should be given, followed in parentheses by a trade or proprietary name or some other accepted designation.

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Crops Research Division
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CONTENTS

1. Crop value and disease
C. E. YARWOOD 755

2. Relationship between climatic factors and incidence of the Sigatoka leaf-spot disease of bananas
L. CALPOUZOS, et al. 758

3. Effect of three viruses on plant production and yields of strawberries
N. L. HORN and R. G. CARVER 762

4. Physiological strains of Heterodera glycines
J. P. ROSS 766

5. Relationships and particle sizes of peach yellow bud mosaic and tomato ringspot viruses
C. H. CADMAN and R. M. LISTER 770

6. Fragaria virginiana, an indicator host for strawberry latent A virus
THOMAS H. KING and DALE A. von RUDEN 772

7. Oospore formation of Aphanomyces euteiches in root tips of Pisum sativum as a method of evaluating resistance
THOMAS H. KING and YONG SUP CHO 777

8. New hosts and characteristics of the Panicum mosaic virus
W. H. SILL, Jr. and LUCIANO T. TALENS 780

9. Downy mildew of peas prevalent in Pacific Northwest in 1962
R. E. FORD and M. J. SILBERNAGEL 784

10. Invasion of and damage to bean seed by storage fungi
LUIS CESAR LOPEZ F. and C. M. CHRISTENSEN 785

11. Losses caused by Rhizoctonia solani borne on bean seed
M. CHORIN and A. HALFON-MEIRI 790

12. Ammonia injury to apples and peaches in storage
E. BRENNAN, et al. 792

13. Viral composition of feathery mottle in Georgia Red sweetpotato
E. M. HILDEBRAND 796

14. An ectoparasitic root nematode on lowbush blueberry (Vaccinium spp.)
G. T. MORGAN and G. W. WOOD 800

15. Effect of date of planting on fungi and other microorganisms isolated from cotton seedlings
HOSNI A. MOHAMED 801

16. Investigation of the ability of Geotrichum candidum and Trichoderma viride to spread by contact infection in nonvented versus vented cartons of lemons
PAUL R. HARDING and DONALD C. SAVAGE 804

17. Apple scald and its control by the use of diphenylamine or Santoquin
ROBERT H. DAINES 808

18. Reduction of aster yellows in flax via systemic insecticides
R. A. FREDERIKSEN 813

19. Ascochyta leaf spot found on three winter oat selections in Kentucky
H. RANDOLPH RICHARDS 818

20. Occurrence of the bean root rot fungus, Fusarium solani f. phaseoli, in Hawaii
E. E. TRUJILLO 819

21. Corrections 819

CROP VALUE AND DISEASE

C. E. Yarwood[1]

Summary

The number of diseases attacking individual crops generally increases with the national value of the crop. For 1960 the minimum number of diseases was 12 for artichoke with a farm value of 3.4 million dollars. The maximum number of diseases was 192 for apples with a value of 195 million dollars. Recorded numbers of diseases have increased about 3 fold between 1926 and 1960. For a 5000 fold increase in the value of all crops, the number of diseases increased about 5 fold.

All crops have diseases, but some crops are more subject to disease than others. The reasons for differences in susceptibility to disease are generally unknown, but many useful correlations have been established. General domestication, single crop culture over large areas, recropping with the same species in the same soil in successive seasons, heavy fertilization, inbreeding, and vegetative propagation usually increase disease. The apparent correlation of crop value and disease seems not to have been pointed out.

Total crop value is a measure of production, but crop production could also be expressed as acreage in cultivation, persons employed in production, gross weight of product, and other criteria. It is believed that the generalization made here would be true whatever criterion was used. Crop values are taken from agricultural statistics for 1959 (3).

The quantitative measure of disease is taken as the number of diseases listed in the check lists of plant diseases for 1926 (1) and 1960 (4). Estimates of crop losses from disease would be a better statistic for this study but such data for individual crops over large areas and extended times are generally not available, and such data on losses as are available are not considered as reliable as the data on numbers of diseases. Many of the individual diseases listed could individually destroy a crop but rarely do.

Numbers of diseases for crops worth over one million dollars are plotted against crop value in Figure 1. The five crops of lowest value were limes, velvet beans, garlic, sweet clover, and eggplant with an average value of 2.1 million dollars. For these crops the average number of diseases listed was 10 for 1926 and 23 for 1960. The five crops of highest value were soybeans, tobacco, wheat, cotton, and corn with an average value of 2.2 billion dollars. For these crops the average number of diseases listed was 26 in 1926 and 86 in 1960. For the 51 crops between these extremes of low and high values the same trend of increasing disease with increasing crop value prevailed.

The fewest diseases listed for 1960 was 12 for artichoke with a value of 3.4 million, and the largest number of diseases was 192 for apples with a value of 195 million.

DISCUSSION

Causes of the correlation of crop value and numbers of diseases are speculative. Superficially, it might be expected, and it is commonly true in localized situations, that the fewer the numbers of diseases and the lower the incidence of each, the greater the crop value. Much effort of plant pathologists is devoted to gathering statistics showing crop losses due to plant diseases (2). Epiphytotics causing severe losses in single crop, season, and locality are usually caused by single diseases, and such incidences are not revealed by the correlation shown here. The basic truths that increased crop production increases disease, and that increased disease decreases production are not in disagreement.

The main reason for the correlation between crop value and disease is considered to be the greater exposure to disease of a high value crop than a low value crop. High crop value is correlated with extensive culture in a variety of environments, where the crop in a variety of physiologic states would be exposed to a variety of endemic diseases. Apples, with the greatest number of diseases, is cultivated in most if not all States of the United States, and in varied environments in most of these. Artichoke, with the lowest number of diseases of any crop, is confined to a few square miles of coastal California. If artichoke culture were increased to the extent of apple culture, it is expected that the disease incidence on artichoke would become

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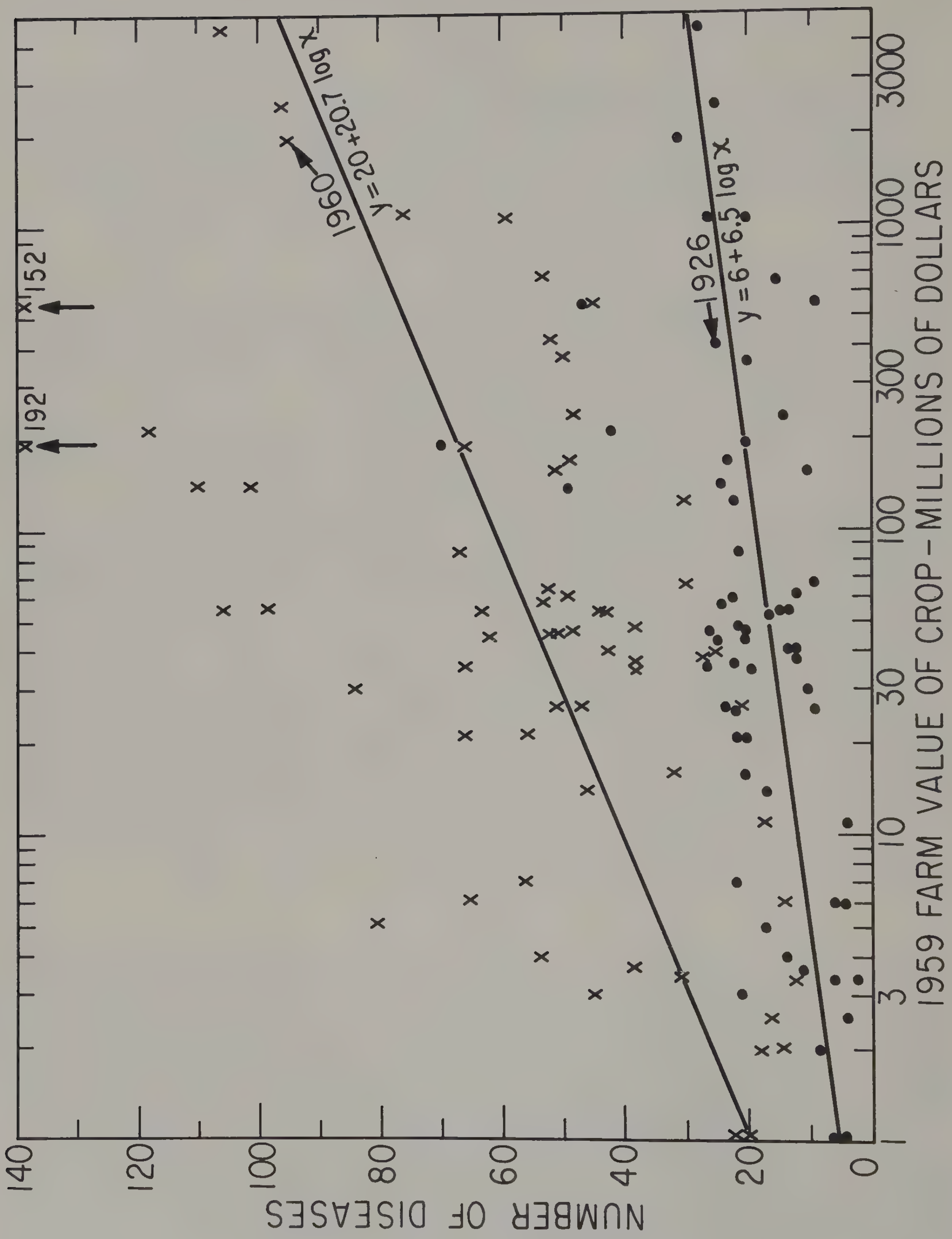

FIGURE 1. Relation of farm value of six crops to numbers of diseases recorded on these crops.

comparable to that on apple. Another reason is that crops of high value are more studied than crops of low value, and thus more diseases are observed and recorded.

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RELATIONSHIP BETWEEN CLIMATIC FACTORS AND INCIDENCE OF THE SIGATOKA LEAF-SPOT DISEASE OF BANANAS

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Summary

A study of the effect of several climatic factors on the incidence of the Sigatoka disease of bananas showed that rainfall data appear to have value for a forecast system. Periods of high rainfall in several locations in Puerto Rico were followed by severe outbreaks of disease. Periods of low rainfall were detrimental to the development of the Sigatoka disease of bananas.

INTRODUCTION

A study of the relation between climate and disease incidence is often a prerequisite to establishing a plant disease forecasting system. Such a system is needed for the Sigatoka disease of bananas, whose pathogen is Mycosphaerella musicola Leach (Cercospora musae Zimm.). The disease is one of the most serious affecting banana plants. It destroys a large amount of leaf tissue and thus causes the plants to produce poor, unmarketable fruit.

Growers who use low-volume oil-based sprays to control Sigatoka apply them once every 2, 3, or 4 weeks, or about 15 to 20 times yearly. This large number of sprays is used to provide continuous protection during the entire year because no satisfactory method of predicting when spraying is necessary is available. The youngest banana leaves are most susceptible, and usually a 4- to 6-week period elapses between inoculation and the appearance of leaf-spot symptoms. Fewer sprays per year would probably be needed to control Sigatoka if a reliable forecast method were available.

It might be possible to establish a Sigatoka-forecast system based on climatological factors. Leach (3) showed that a seasonal variation in disease incidence occurs in Jamaica. Moderate temperatures and high moisture are known to be important for the sporulation, germination, and growth of the causal fungus (1, 3). Recent reports from Guadeloupe and the Cameroons (2, 4) suggested that temperature and relative humidity data or rainfall and sunshine data would be useful for predicting Sigatoka epiphytotics. Further studies on climatic conditions from other banana-growing areas are needed.

In the present investigation, data were obtained for 2 1/2 years on Sigatoka disease incidence, temperature, relative humidity, rainfall, and wet-foliage periods at three climatologically different locations in Puerto Rico. These data were examined to determine whether a relation exists between Sigatoka disease incidence and any of the weather factors studied.

MATERIALS AND METHODS

Continuous records for each climatological factor were kept. The data on temperature, relative humidity, and periods of moisture on leaf surfaces were obtained by using an instrument described previously (5). The instrument consists of an ordinary hygrothermograph connected to a special pencil that records on a glass plate only when the surface is wet from rain or dew. Rainfall data on a daily basis were received from the U. S. Weather Bureau, which had rain gages in the vicinity of each of the three observation sites.

Sigatoka disease incidence was observed each week on Dwarf Cavendish banana plants. Banana plants produce leaves one at a time about every 7 to 14 days during the entire year until the fruit stalk appears, at which time leaf production ceases. The percentage leaf area destroyed by the disease was recorded on three mature, leaf-producing plants at each site. As new leaves developed, they too were observed. When the fruit stalk appeared on a plant, another plant would be substituted so that three leaf-producing plants might be kept under continuous weekly observation. In this way, a year-round record of disease development was obtained. The experiment began in February 1956 and ended in September 1958.

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DESCRIPTION OF SITES

The three sites were located in western Puerto Rico. The location of each site, its average annual rainfall, its altitude above sea level, and the total banana clumps were as follows: Mayaguez 80 inches, 30 feet, 100 clumps; Lajas (Palmarejo) 44 inches, 100 feet, 150 clumps; Monte del Estado 102 inches, 2900 feet, 20 clumps. The plants were already established at each site before this experiment was set up. No spray for Sigatoka control had been applied in this region of Puerto Rico; therefore, the plants at all sites were under natural disease conditions.

RESULTS

All the data for each site were summarized on a 3-week basis and plotted as line graphs (Figures 1-3). The disease curve shows the average percentage of leaf tissue destroyed by Sigatoka on three plants. The rainfall curve is based on inches of rain. The temperature curve indicates the mean temperature during each 3-week period. The relative humidity curve gives the percentage of time when relative humidity was 98% or higher. The wetness curve shows the percentage of time when the leaf surfaces were wet.

The disease curve for Mayaguez (Fig. 1) shows distinct annual cycles, with minimum levels during the dry season in the spring and early summer of '56, '57, and '58, and maximum levels during the wet seasons in the fall of '56 and '57. In Figures 2 and 3, Lajas and Monte del Estado, respectively, the disease curves are not as clearly cyclic as in Figure 1. Disease at Lajas was at a minimum in the spring of '56 and built up to a maximum in the fall of the same year. A prolonged general decline which bottomed out in the fall of '57 followed. A shallow rise in disease extended from the fall of '57 to the spring of '58; a decline followed and then a new rise started. Disease incidence on Monte del Estado rose from a minimum in the spring of '56 to a maximum in the fall, followed by a general decline which bottomed out in the summer of '57. A maximum occurred in the fall of '57, after which the disease declined slowly to a minimum in the spring of '58; a slightly higher but irregular trend followed. At all sites a higher Sigatoka disease incidence occurred during 1956, a normal wet year, than in 1957, a dry year.

The rainfall curve for Mayaguez (Fig. 1) shows distinct annual cycles that precede similar trends in disease incidence. By an empirical examination of the graph, it seemed that there was a minimal rainfall level (3 inches per 3-week period) above which the curve coincided with periods when disease was either rising or was at a high level. When the rainfall curve fell below this minimal level, the disease was generally declining. At Lajas, during 1956, there were parallel trends between rainfall and disease. This is more evident when a rainfall above 3 inches per period is noted. In 1957-58, data showing the trends between rainfall and diseases were not striking, possibly because the weather was unusually dry. The data from Monte del Estado contained a reasonably good match in trends between rainfall and disease curves. This is clearer when rainfall above 6 inches per period is noted. The 1957-58 season had distinct cycles of rain; however, the disease cycles were much less marked, again possibly due to drier conditions that prevailed. Nevertheless, in general, both curves have similar trends. At all three sites there is a tendency for the rainfall curve to lead the disease curve, usually by about 1 to 2 months.

The temperature curve at Mayaguez seems to precede the disease curve during most of 1956; however, there is no parallelism after that period. At Lajas there is no apparent relation between the temperature and disease curves. At Monte del Estado, the former curve precedes and parallels the other during 1956, but not thereafter.

Relative humidity at Mayaguez seems to have annual cycles that forecast the disease cycle during 1957-58. This relation, however, was not as clear during 1956. At Lajas and Monte del Estado, there is no general parallelism between relative humidity and the disease cycle.

The curve for wet leaves (from dew or rain) at Mayaguez also is cyclic and precedes the trends found in the disease curve. Wet-leaf data for Lajas and Monte del Estado parallel disease only during the first half of 1957. Unfortunately, these wetness curves have large gaps for 1956, making it difficult to evaluate the data.

DISCUSSION AND CONCLUSIONS

A forecast system for Sigatoka control should be sufficiently convenient to be used by individual banana growers. Disease forecasts based on climatic conditions cannot be made for

Fig. 1

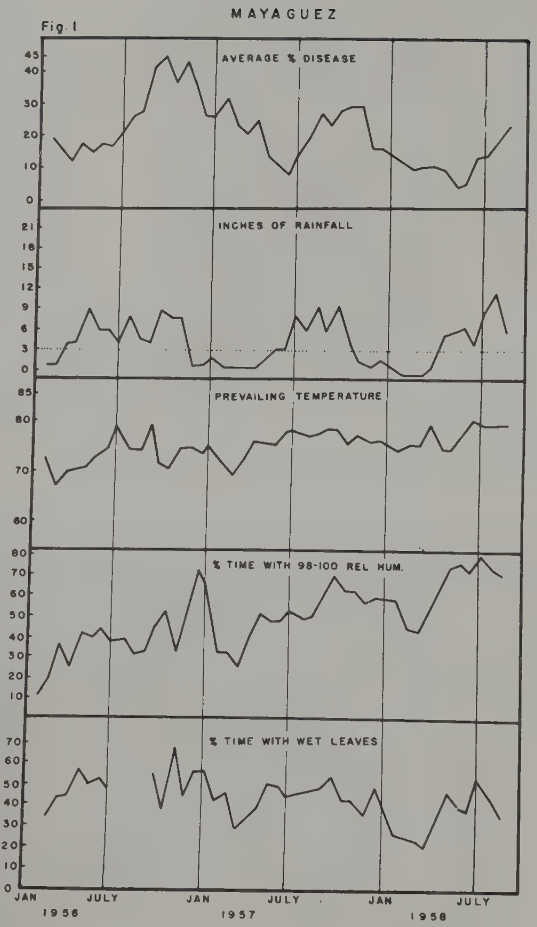


Fig. 2

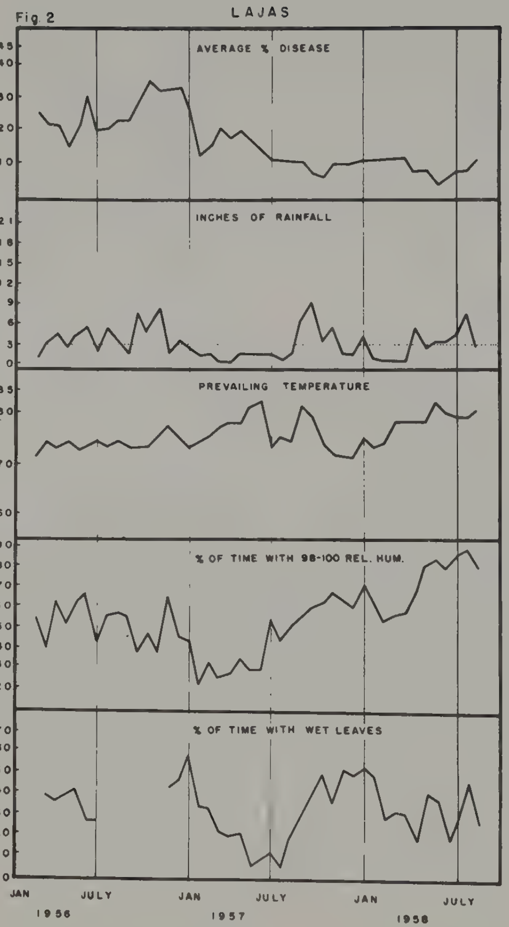
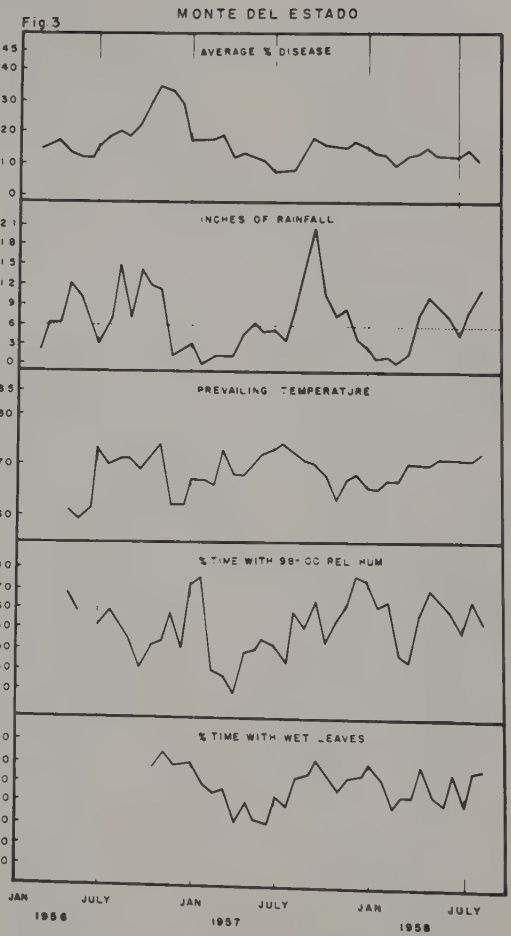


Fig. 3



FIGURES 1-3. Incidence of Sigatoka disease and associated weather at three climatologically different sites in Puerto Rico. The curves are based on data points representing the average for 3-week periods, from January 1956 to September 1958.

broad tropical areas because of the large variations in the weather over short distances. Each grower, therefore, probably will have to make his own decisions when to spray. A useful forecast system should meet the following conditions: 1) collection of data should involve inexpensive, accurate equipment easy to maintain; 2) the data should be easy to interpret and not require involved calculations; 3) the predictive value of the data should be fairly reliable, perhaps around 80%; and 4) the forecast system must permit those depending on it to reduce the current number of spray applications without losing disease control.

Several climatic factors interact to affect disease incidence with almost all plant diseases. If a forecast system can be based, however, on one factor only, the evaluation of data would be simplified. Each climatic factor in this study did at times predict the disease trend. However, the rainfall curves for the three sites were more closely related to the disease curves than were the other factors. Even here there were discrepancies. The Mayaguez rainfall curve showed the clearest relation to disease. The Lajas curve was less obvious during the unusually dry period of 1957-58. The Monte del Estado rain curve was more apparent than Lajas. However, cooler temperatures probably affected disease incidence at Monte del Estado, since it had the highest rainfall of the three sites but less disease than Mayaguez.

The data indicate that in at least some geographical locations amount of rainfall may be useful for predicting future trends in Sigatoka disease incidence. Rainfall has advantages of requiring only the simplest instrument for measurement -- a rain gage -- and the accumulated data on a 3-week basis can be used directly without further calculations. The graphs suggest that disease control may require spray applications only when rainfall exceeds a minimal rate. This rate at the two sea level sites was around 3 inches per 3-week period.

The data obtained in this experiment are useful as a first step to establish a Sigatoka spray forecast system because they reveal possible relation between certain weather factors and disease incidence under different climatological conditions. Although the results of these trials point toward the use of rainfall in a spray forecast system, they do not prove that rain data alone will be a practical criterion. More conclusive evidence must come from a field-spray program which uses a Sigatoka forecast system based on rainfall.

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EFFECT OF THREE VIRUSES ON PLANT PRODUCTION AND YIELDS OF STRAWBERRIES

N. L. Horn[1] and R. G. Carver[2]

Summary

Three viruses were commonly found in Louisiana commercial strawberry plantings, namely, mottle virus (MV), vein banding virus (VBV), and aster yellows virus (AYV). An apparently undescribed virus was found in one location on an unknown strawberry variety. It was given the name chlorotic fleck virus (CFV) on the basis of symptom expression in two Fragaria vesca clones. Commercial varieties were over 80% infected with MV and 6% with VBV. AYV was found occasionally but was not of economic importance. Plant production and fruit yields were significantly reduced when healthy plants of the var. Headliner were artificially inoculated with MV, VBV, and CFV.

INTRODUCTION

The literature dealing with the effects of viruses on fruit yield and plant production of strawberries is greatly limited. Plakidas (12) estimated an average of 50% decrease in productiveness of the strawberry plantings in the coastal region of central California at the time the Marshall variety was the principal commercial variety grown. Zeller (14) showed that yields of 2-year crinkle-infected Marshall plants were 52% less than yields of healthy plants. Stitt and Breakey (13) stated that by 1946 yellows had become the limiting factor in strawberry production in the Pacific Northwest in the susceptible Marshall variety with decrease in yields as high as 75%.

More recently fruit yields and plant production from virus-free plants were compared with virus infected stocks of various varieties of strawberries (1, 2, 5, 7, 8, 9, 10, 11). Observed differences have generally been in favor of the virus-free stock. None of these reports indicated, however, that the virus-free and infected plants used in these tests were from stock with a common history. Carver and Horn (4) compared fruit yields and plant production of virus-free plants with artificially inoculated plants with a common history. This method was used also in the work presented here so that a more accurate measure of the effect of the viruses on yields of fruit and plants was obtained than previously described.

RESULTS

A survey was made to determine what viruses occurred in the commercial strawberry areas of Louisiana during the years 1960-1962. Scions were collected from Klonmore, Headliner, Dabreak, Klondike, Marion Belle and Konvoy varieties and grafted by the method described by Bringhurst and Voth (3) to Fragaria vesca indicator clones EMM, which contained a mild strain of latent A virus, EMK, which was apparently free of virus, and BEM, which contained a severe strain of latent A virus. MV and VBV were the most frequent ones found. AYV was found occasionally. An apparently undescribed virus was isolated in one location from plants of an unknown variety. MV and VBV were the only ones considered to be of economic importance as a result of the survey because they were found universally in all Louisiana varieties. The occurrence of MV and VBV in Louisiana strawberry varieties which were obtained from 22 farms is shown in Table 1.

Additional indexing was made from plants grown at the Louisiana State University Experiment Station in the strawberry breeding program. Among those indexed for virus were commercial varieties obtained from Louisiana plant growers as well as seedlings and various selections. The occurrence of MV and VBV found in this planting is given in Table 2.

Identifications of MV and VBV were made on the basis of symptom expression in the F. vesca clones, as described in the literature with particular attention directed to the work of Frazier and Posnette (6), and on insect transmission studies. The insect vectors used were Aphis gossypii Glover, Myzus porosus Sand., and Macrosiphum rosae (L.) collected in Louisiana commercial plantings. This is believed to be the first report of M. rosae in Louisiana.

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Table 1. The occurrence of MV and VBV in Louisiana strawberry varieties.

Variety	No. grafts made	No. indicator clones to express symptoms of		Total % infection
		MV	VBV	
Headliner	132	103	8	84
Dabreak	23	22	0	95
Klondike	36	31	3	95
Klonmore	56	45	6	91
Marion Belle	31	26	1	87
Konvoy	12	4	3	58

Table 2. The occurrence of MV and VBV in commercial varieties, seedlings, and selections at the Louisiana State University Experiment Station Farm.

Variety, seedling or selection	Year grown at L. S. U.	No. grafts made	No. indicator clones to express symptoms of		Total % infection
			MV	VBV	
Headliner	1	6	5	1	100
Klonmore	1	6	6	0	100
Marion Belle	1	6	5	0	84
Konvoy	1	6	6	0	100
Calif. Sel. 4245	2	6	6	0	100
0-621	1	4	4	0	100
0-1158	1	4	3	0	75
0-1080	1	3	2	0	66
0-563	1	4	3	0	75
0-1968	2	4	4	0	100
9-403	2	4	3	0	75
9-1881	2	4	3	1	100
5-325	5	6	5	1	100
5-264	5	4	3	0	75
7-42	10	6	4	2	100

Table 3. Plant yields from healthy and MV infected seedlings.

Seedling no.	Treatment	
	virus-free	mottle
788	288	129
5325	571	110

Table 4. Mean plant yields per plant from healthy and virus-infected Headliner plants.

Virus-free	VBV	MV	CFV	Commercial complex
35.2	11.2	12.8	10.8	3.0
LSD 5%	8.5			
1%	11.4			

A. gossypii and *M. porosus* were vectors of MV but not VBV. The latter was the more efficient vector. *M. rosae* was a vector of VBV but not MV. *Myzus persicae* Sulzer, *Aphis forbesi* Weed, *Macrosiphum euphorbiae* (Thomas) and *Cerosipha rubifolii* failed to transmit MV or VBV.

Table 5. Average fruit yields in crates per acre from healthy and virus-infected Headliner plants.

Reps	Virus-free	VBV	MV	CFV	Commercial complex
1	262	201	202	184	109
2	251	241	168	203	190
3	297	236	172	197	142
Means	270	226	180	194	147
LSD	43.8 61.0				

The apparently undescribed virus was obtained from an unidentified strawberry variety collected outside the commercial area. Scions were grafted to EMK and BEM indicator plants. In 15 days the BEM plants developed symptoms different from those of MV or VBV. Leaves that were 3/4 grown were downcurled with yellow margins. Newer leaflets developed unevenly. There was usually a slight vein clearing which faded in 7 to 10 days at which time numerous chlorotic islands appeared on the younger leaflets. The chlorotic flecking faded in 3 to 4 weeks so that the chlorosis and dwarfing were not permanent. On EMK the symptoms were less pronounced, the leaves being very slightly downcurled with faint vein clearing. The chlorotic pattern developed in much the same manner, then faded in 3 to 4 weeks. The final symptoms on BEM and EMK plants resembled those produced on F. vesca by heat, but were graft transmissible. For convenience the isolate will be referred to here as chlorotic fleck (CFV).

In March 1960, scions from greenhouse grown Louisiana seedlings 5325 and 788 were grafted to BEM plants and found to be virus-free. Scions from MV infected EMK plants were grafted to half of each of the two seedlings. After 4 weeks the inoculated seedlings were indexed to be sure that the MV was transmitted to them. In July, eight infected plants and eight healthy plants of each seedling were planted side by side in the field under separate cages to study the effect of MV on plant production. The plants were set on rows 6 feet wide in which the soil had been treated previously with methyl bromide at the rate of 1 pound per 100 square feet. The runner plants were dug and counted December 1, 1960, the results of which are recorded in Table 3. The virus-free plants of both seedlings were more vigorous than the virus-infected plants. Plant yields of healthy seedling 788 were more than twice those of the virus-infected ones while plant yields of seedling 5325 were five times greater than those of the corresponding infected ones. These plants were placed immediately in a randomized block to determine the effect of mottle on fruit yield. The plots were 20 feet long with four replications, each contained 20 plants on rows with 3.5-foot centers with 3-foot spacings between plots along the rows. All plants were sprayed with demeton 26.2% (Systox) until blooming period began. During March, April, and May the fruit yields were recorded. Those from virus-free seedling 5325 were highly significant over mottle-infected ones. No significant difference was found in the case of seedling 788.

In July 1961, known virus-free Headliner plants, artificially inoculated with MV, CFV, and VBV respectively, virus-free Headliner plants, and commercially grown Headliner plants, which were found to contain at least one of the above viruses, were planted under cages in the field as described in the 1960 test. The number of plants produced in each treatment, recorded November 30, 1961, are shown in Table 4.

The production of virus-free plants was significantly higher than all other treatments at the 1% level. Samples of plants from each of these treatments were placed immediately in a fruit yield test similar to the one in 1960. During March, April and May of 1962 the yields were recorded in terms of crates per acre. The data in Table 5 indicate the three viruses have a significant effect on yields of berries. They also show that the yields of commercial Headliner plants are greatly affected by the complex of viruses which they contain.

DISCUSSION

The effect of viruses on fruit yield and plant production of strawberries was measured more accurately by the method used here than those previously described. First, all the plants used in yield trials in these tests were from stock with a common history. They were grown side by side under similar conditions except for the presence or absence of virus of a known source. Furthermore, the plants were grown in methyl bromide treated soil under screened cages while in the plant production tests. When transplanted in fruit yield tests, they were exposed to insects from December to May. Even then the plants were treated chemically, however, until April 1, so that virtually no aphids were found on the plants during the fruit yield tests. Also, the plants were isolated from other strawberry plantings by a distance of not less than 3 miles.

From the virus survey made in Louisiana the commercial plantings were probably greater than 90% infected. This was not surprising because a survey of the plants used in the breeding program at the Louisiana State University, the original source of new varieties for commercial growing, were at least 90% infected. At the present time the Dabreak and Headliner varieties are by far the leading ones grown in the State. Both were products of this breeding program and, as far as is known, all the stocks for commercial plantings of these two varieties came directly from the University farm. Even seedlings that were set in the field for the first year contained a very high percentage of infection (Table 2).

Furthermore, high populations of vectors were found in many of the commercial plantings during March and April. An aphid survey which was not presented here but which was made in connection with this report, showed that Myzus porosus, and Aphis gossypii were found universally in the commercial area. This would have accounted possibly for the high incidence of MV infection. A low incidence of VBV may have been attributed partly to the scarcity of Macrosiphum rosae.

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PHYSIOLOGICAL STRAINS OF HETERODERA GLYCINES

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Summary

Populations of Heterodera glycines from North Carolina and Tennessee were compared for ability to develop on a soybean line rated resistant in Tennessee and susceptible in North Carolina and on a variety rated susceptible in both States. In two experiments, females of the Tennessee population rarely developed in the resistant soybean line while female development of the North Carolina population was not inhibited. On the nineteenth day after inoculation over 50% of the Tennessee nematode population remained in the second and third stages (35% were obviously degenerate) in the roots of the resistant line, while only 14% of the nematodes from North Carolina remained in these stages of development. Female development of both populations were equal in the susceptible variety. These data demonstrate the existence of physiological specialization of H. glycines.

INTRODUCTION

Resistance of soybeans to the soybean cyst nematode, Heterodera glycines Ichinohe, was first recorded in North Carolina in 1957 when four soybean lines were found that precluded development of female nematodes (2). Later these symptoms were found to be resistant in Tennessee, and additional lines were also classified as resistant[2]. Some of the Tennessee resistant lines were previously classified as susceptible in North Carolina, suggesting that the populations of H. glycines in the two States differed in their pathogenic abilities. This paper reports the results of investigations designed to determine whether physiological strains of H. glycines exist.

MATERIALS AND METHODS

A population of H. glycines from Tennessee (TCN) was obtained from J. M. Epps and compared with a population of H. glycines from New Hanover County, North Carolina (NCN). Seed of soybean Plant Introduction (P. I.) 88788, classified as resistant in Tennessee and susceptible in North Carolina, was obtained from the U. S. Regional Soybean Laboratory, Urbana, Illinois. This plant introduction and the soybean variety Lee, susceptible to both populations of H. glycines, were used as test plants.

Two experiments were conducted in which standardized nematode inocula were used. In the first experiment, conducted during the winter of 1960-61, cysts from greenhouse cultures of the nematodes were selected for similarity in size and age. Twelve cysts from NCN were placed in each of 16 Syracuse watch glasses and crushed to enhance hatching; cysts of TCN were similarly treated. The contents of each watch glass were washed into a depression made in the soil in a 3-inch pot, and one pre-germinated soybean seed was planted per pot. Half the pots containing each nematode culture were planted with Lee and the other half with P. I. 88788. The pots were maintained in the greenhouse where temperatures often were unusually low (45° to 55° F). After 33 days the soil was washed from the roots of each plant and loose cysts recovered on a 60-mesh sieve. Males were not counted. Since few cysts were recovered and few females found on the roots, each plant was maintained separately in nutrient solution. After 2 weeks, the solution from each plant was screened through a series of 30-, 80-, and 200-mesh sieves. After each root system had partially air-dried, it was supported on the sieves and forcibly sprayed with water to dislodge any adhering nematodes. Females and males were retained on 80- and 200-mesh sieves, respectively. All recovered nematodes were counted under the dissecting microscope.

The second experiment was conducted 1 year later in the greenhouse during the winter. This experiment was basically the same as the first but was modified to include standardized larval suspensions for inocula, thorough examinations of the infected roots, and attempted recovery of all adult nematodes. The inocula in this test consisted of second-stage larvae from cultures propagated in microplots during the previous summer and were prepared by blending the cysts for 2 minutes. The resulting suspension was placed on a muslin cloth resting on a shallow layer of water. During the next 4 days larvae were collected twice daily and stored at 50° F. Suspensions containing 15 larvae per ml were prepared from each population.

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Uniform soybean seedlings of Lee and P.I. 88788 grown in 1 1/2-inch pots were selected when the first trifoliolate leaves were expanding. A depression was made with a 1 1/2-inch pot in the soil in 48 3-inch pots. The soil in half the pots was infested with NCN and in the other half with TCN by pipetting 13 ml of larval suspension into the depression in each pot. Half the pots infested with each nematode population were planted to Lee seedlings and half to seedlings of P.I. 88788. The plants were grown in a greenhouse at temperatures of 75° to 80° F.

The root systems of four plants in each treatment were washed out and examined after 19 days' incubation to ascertain the status of nematode development. The male nematodes in the soil of each pot were retained on a 200-mesh sieve and placed in a Baermann funnel. After 1 week the males were counted. No female nematodes were found in the soil at this time. The washed root systems were stained with cotton blue-lactophenol for 2 hours at 50° to 60° C and cleared with lactophenol. The entire root systems were examined under the dissecting microscope and the condition and stage of development of the nematodes were recorded.

Four weeks after inoculation, roots of the remaining plants were washed free of soil. The females in each soil suspension were recovered on a 60-mesh sieve and the males were recovered as above. To recover the females attached to the roots, each root system was allowed to air dry for 5 minutes and then was forcibly sprayed against a 60-mesh sieve. These nematodes were also counted.

Comparisons of individual treatments were made by computing the standard error of the difference for each comparison from the individual treatment errors. The analysis of variance was not used since there was heterogeneity in the errors of the various treatments.

RESULTS

First Experiment: The average number of TCN females recovered per plant of Lee was 15 times the number recovered per plant of P.I. 88788 (Table 1). On the other hand, the average number of NCN females recovered from Lee was roughly four times greater than the number recovered from P.I. 88788. These data indicate that NCN is more capable than TCN of reproduction on P.I. 88788.

Table 1. Adult males and females of *H. glycines* from Tennessee and North Carolina populations recovered from soil and removed from roots of P.I. 88788 and Lee soybeans after 47 days[a].

Treatment	Source of nematode	Soybean	Males	Females[b]
A	Tennessee	P.I. 88788	4	1
B	Tennessee	Lee	2	15
C	North Carolina	P.I. 88788	5	17
D	North Carolina	Lee	21	63
Statistical comparison of treatments[c]			A-D** B-D** C-D**	A-B** A-C** A-D** B-D* C-D*

[a] Average number nematodes from eight plants.

[b] Includes females recovered after 33 days.

[c] * and ** denote statistical differences at the 5% and the 1% level, respectively, between the two treatments designated.

The difference between the total number of TCN and NCN adults recovered from the susceptible Lee plants indicates the inocula of the two nematode isolates were not of equal potential although equal number of cysts were used in the inocula. Since males were neither recovered nor counted when the roots were washed out 33 days after planting, all male counts recorded are erroneously low.

Table 2. Stages of development of North Carolina and Tennessee populations of H. glycines in roots of P.I. 88788 and Lee soybeans 19 days after transplanting to infested soil[a].

Treatment	Source of nematode	Soybean	% Degenerate larvae[b]	% Second and third stage larvae	% Fourth stage and adult: Males	Females	Total no. nematodes
A	Tennessee	P.I. 88788	35	53	44	3	133
B	Tennessee	Lee	1	4	71	25	127
C	North Carolina	P.I. 88788	10	14	65	21	179
D	North Carolina	Lee	7	9	68	23	173
Statistical comparison of treatments[c]			A-B** A-C* A-D* B-C* B-D*	A-B** A-C** A-D** B-C*	A-B* A-D*	A-B** A-C** A-D**	N.S.

[a] Average of four plants per treatment.

[b] Most degenerate larvae were in second and third stage.

[c] * and ** denote statistical differences at the 5% and 1% level, respectively, between the two treatments designated.

Second Experiment: Since most of the second- and third-stage larvae found in the roots 19 days after inoculation showed signs of degeneration, the data for larvae in these two stages were combined for statistical analyses (Table 2). The fourth-stage male and female larvae never exceeded 8% of the total nematodes observed in a treatment and were grouped with adult males and females, respectively, for statistical analyses.

The stages of development of the NCN and TCN nematodes within the roots of the two soybean lines 19 days after inoculation revealed significant differences in nematode-host interaction (Table 2). Females of TCN rarely developed in roots of P.I. 88788 whereas females of NCN developed to the same degree on P.I. 88788 as they did on Lee. Female development of TCN and NCN on Lee was similar. Male development of TCN on P.I. 88788 was significantly less than male development on Lee; NCN showed no such difference.

The large percentage of degenerate second- and third-stage larvae present in roots of P.I. 88788 infected with TCN was associated with the large percentage of larvae remaining in these stages of development. In Lee, larval degeneration of TCN was less than that of NCN.

There was no significant difference in the total number of nematodes found in the roots of the four treatments 19 days after inoculation. This indicates the two inocula were equally infective.

The difference between the two nematode populations was also evident from the recovery data of adult males and females at the termination of the experiment (Table 3). The number of adult females of TCN recovered from roots of P.I. 88788 was extremely low compared with the adult females recovered in the other three treatments. The number of NCN adult females recovered from P.I. 88788 was less than the number recovered from Lee; however, the magnitude of this difference was much smaller than that found with TCN. More males of NCN than of TCN were recovered from Lee.

DISCUSSION AND CONCLUSIONS

The standardized larval suspensions used in the second experiment produced more uniform infection than the inocula derived from equal numbers of cysts employed in the first experiment. The source of the cysts used to supply the larval suspensions may also have added to the uniformity of the inocula. The use of uniform soybean seedlings in the second experiment probably provided for more similar numbers of entry sites than did the germinated soybean seeds used in the first experiment. While the results of the first experiment indicated that NCN females possessed greater ability to reproduce on P.I. 88788 than did TCN females, the magnitude of variation throughout the experiment prompted the second more closely controlled experiment.

In the second experiment, more males and females were found in the roots 19 days after inoculation than were recovered from the soil and root systems at the termination of the experiment. This discrepancy could reflect either a failure of some adults to emerge from the

Table 3. Adult males and females of H. glycines from Tennessee and North Carolina populations free in soil and removed from roots of P. I. 88788 and Lee soybeans 28 days after transplanting to infested soil.

Treatment	Source of nematode	Soybean	Males	Females
A	Tennessee	P. I. 88788	7[a]	1
B	Tennessee	Lee	8	24
C	North Carolina	P. I. 88788	12	15
D	North Carolina	Lee	21	26
Statistical comparison of treatments[b]			A-D**	A-B**
			B-D**	A-C**
				A-D**
				B-C*
				C-D*

[a] Average of eight plants per treatment.

[b] * and ** denote statistical differences at the 5% and 1% level, respectively, between the two treatments designated.

roots, or an inefficiency in the methods used to recover the adults. This is most noticeable with respect to the males.

The large number of TCN second- and third-stage larvae (53%) found in the roots of P. I 88788 after 19 days in the second experiment indicates that this host is not suitable for TCN. Besides the 35% degenerate TCN larvae in the roots of P. I. 88788, it is highly probable that many of the normal appearing TCN second-stage larvae found after 19 days would have eventually died. This idea is supported by the relatively few TCN adults recovered from the roots of P. I. 88788 after 28 days.

The ratio of male to female fourth-stage larvae and adult nematodes found in the roots after 19 days was 3:1 in all treatments except TCN in roots of P. I. 88788 where the ratio was 15:1. Histological studies of a North Carolina population of H. glycines in roots of highly resistant soybeans revealed that only males developed (1). Therefore, based on sex ratios, P. I. 88788 behaved as a resistant host when infected with TCN and as a susceptible host when infected with NCN. The large preponderance of males probably is directly related to the degeneration of second- and third-stage female larvae in the resistant host. The difference in sex ratios on the differential host reflects a physiological difference between the two nematode populations.

That P. I. 88788 possesses some resistance to the NCN is shown in the data collected at the termination of the second experiment since fewer females were recovered from P. I. 88788 than from Lee. However, the degree of this resistance in no way approaches that displayed by P. I. 88788 toward the TCN.

The extremely low percentage (1%) of degenerate TCN larvae found in the roots of Lee indicates that larvae of this nematode population are as successful in the roots of a susceptible plant as those of the NCN. The similarity in the average number of adult TCN females and NCN females found on Lee at the end of the second experiment supports this point.

Since the success of the female of H. glycines to develop and produce eggs determines whether the nematode will maintain itself on a given host, the number of adult females that develop from a standard nematode inoculum is a measure of host resistance to the nematode. Consequently, different physiological strains of H. glycines exist if two nematode populations manifest significant differences in female development on a given host when the test is performed under uniform conditions.

The results presented show that TCN is physiologically different from NCN and that this difference may be demonstrated on P. I. 88788 by the relatively sparse development of females of the Tennessee population as compared with the development of the North Carolina population.

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RELATIONSHIPS AND PARTICLE SIZES OF PEACH YELLOW BUD MOSAIC AND TOMATO RINGSPOT VIRUSES

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Abstract

Two isolates of tomato ringspot virus (TRSV) infected and caused symptoms in peach indistinguishable from those caused by a Californian isolate of peach yellow bud mosaic virus (PYBV). Measurements on phosphotungstate-treated preparations showed that the particles of PYBV and TRSV are similar and close to 25 mμ diameter. These results confirm those from earlier serological tests which showed PYBV is indistinguishable from TRSV.

Elsewhere (3) we reported evidence from plant protection and serological tests which led us to identify peach yellow bud mosaic virus (PYBV) with tomato ringspot virus (TRSV). Although we made the attempt, we failed to show that our isolates of TRSV infected and caused yellow bud mosaic symptoms in peach. We also claimed that the particles of PYBV were around 25 mμ diameter in contrast to Weintraub's (10) estimate of 45-50 mμ which appeared when our paper was in press. Frazier, et al. (4) implied that these discrepancies might invalidate the identification of PYBV and TRSV. This implication was disturbing for several reasons, and led us to make the following experiments with the Cornell and Wisconsin isolates of TRSV and the Californian isolate of PYBV described in our previous paper.

SYMPTOMS IN PEACH

The viruses were propagated in Petunia hybrida, and sap from newly systemically infected leaves used to inoculate batches of 1-month-old peach seedlings that had been kept heavily shaded for the previous 2 days. Three weeks later plants inoculated with each of the three virus isolates developed symptoms. Although the three isolates differ somewhat in the symptoms they cause in some kinds of herbaceous plants (3), all caused similar symptoms in peach and these agreed with published descriptions of yellow bud mosaic disease. Viruses reisolated from infected peach seedlings caused characteristic symptoms in herbaceous test plants, and serological tests made with heat-clarified saps from these failed to detect any antigenic differences between the three virus isolates.

PURIFICATION AND ELECTRON MICROSCOPY

When in Petunia sap, PYBV and TRSV are, as we found earlier, readily destroyed by chemicals that have little or no effect on other viruses of the tobacco-ringspot type. Methods of clarifying sap by heating or by mixing with n-butanol, butanol/chloroform, or ether were all much less successful in making preparations fit for electron microscopy than one based on the method used by Senseney, et al. (8) for purifying TRSV. Newly, systemically infected Petunia leaves were first weighed and minced, and the pulp then squeezed through muslin after mixing with 3% by weight of K_2HPO_4 dissolved in a little distilled water. Two successive cycles of differential centrifugation in a Model L Spinco ultra-centrifuge (30 minutes at 15, 000 g followed by 2 hours at 70, 000 g) yielded preparations which, although not entirely free from cellular debris, were adequate for electron microscopy.

Samples of such preparations were mixed with 1% phosphotungstic acid adjusted to pH 7 with NaOH, mounted on carbon-coated grids and photographed with a Siemens Elmiskop I electron microscope, using double condenser illumination and an instrument magnification of 40, 000X at 60 k. v. For comparison, a preparation of an English isolate of arabis mosaic virus, purified by the butanol/chloroform method of Steere (9) was photographed under the same conditions. The negatives were viewed with a cathetometer and measurements (two diameters at right angles) made on a random sample of 25 particles of each virus. The particles in all the electron micrographs were very uniform, and there was no evidence of distortion or of the flattening observed by Senseney, et al. (8) in metal-shadowed preparations of TRSV. As shown in Table 1, the diameters of the particles of PYBV and the two isolates of TRSV were very similar and close to our original estimate of 25 mμ. The figure for arabis mosaic virus is within the range found by Harrison and Nixon (6).

Table 1. Dimensions of virus particles.

Virus	Mean diameter (in mμ): (25 particles)	Standard error
Peach yellow bud mosaic	26.6	0.2
Tomato ringspot (Cornell)	26.7	0.2
Tomato ringspot (Wisconsin)	25.8	0.4
Arabis mosaic	27.6	0.2

DISCUSSION

As Harrison and Nixon (6) point out, the apparent diameter of a spherical virus particle varies with the treatment given the specimen. We think this accounts for the discrepancy between our figures and those of Weintraub (10) for PYBV (45-50 mμ) and of Senseney, et al. (8) for TRSV (43 x 13.5 mμ), each of whom examined metal-shadowed preparations. Other evidence suggests that the particles of PYBV and TRSV are more fragile than those of other viruses of the tobacco-ringspot type, and they may be more subject to distortion by exposure to the temperature at which uranium volatilizes. Senseney, et al. suspected that the pill-shaped particles they observed might have been distorted, and estimated that spherical particles of equivalent volume would have a diameter of 27 mμ, a figure close to ours for TRSV.

There seems no evidence therefore to conflict with the idea that peach yellow bud mosaic is a disease of peach caused by tomato ringspot virus.

Because of genetic variation in both host plant and virus, differences among serologically indistinguishable isolates of TRSV in ability to infect peach would not have been surprising. The point at issue is the weight attaching to such differences and the use of the word 'strain' where perhaps 'isolate' or 'variant' would be more appropriate. At present, serology provides the best evidence of relationship between viruses and this must take precedence over other kinds of evidence if any logical classification of viruses is to evolve.

Where it can be shown that viruses share different proportions of antigens, as with different strains of tomato black ring and raspberry ringspot viruses (1, 5), one is entitled to speak of 'strains.' Distantly related viruses, like arabis mosaic and grape vine fanleaf viruses (2), are, as proposed by Kassanis (7), best regarded as representatives of different serotypes of one virus. Our results (3) showed that PYBV is serologically indistinguishable from an earlier-described virus, TRSV, and is therefore best regarded simply as an isolate or a variant of TRSV. The fact that individual isolates or variants of a virus differ in symptomatology or host range is largely irrelevant in face of the evidence of their serological relationship.

We wish to thank Mr. J. Cathro for making the electron micrographs.

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FRAGARIA VIRGINIANA, AN INDICATOR HOST FOR STRAWBERRY LATENT A VIRUS[1]

Thomas H. King and Dale A. von Ruden[2]

Summary

A clone of Fragaria virginiana collected in northern Minnesota is an effective indicator host for determining the presence of latent A in strawberry. The virus causes epinasty, dwarfing, veinal necrosis and deformation of leaflets in various degrees in F. virginiana. Mature, fully developed leaves are also affected by the virus. The host disclosed strains of latent A and was more effective in demonstrating the presence of mild strains of latent A than other methods now being used.

The East Malling clone of Fragaria vesca is the indicator plant most widely used for indexing strawberry stocks for the presence of viruses. Other selections and clones of this species and other wild species of Fragaria have also been used for virus indexing (1, 5, 7).

In 1953, Frazier (2) reported the discovery of a latent virus in Fragaria vesca and F. bracteata. He designated the virus from F. vesca (East Malling clone) as latent A and that from F. bracteata as latent B.

In 1958, McGrew (4) stated that all stocks of the East Malling clone (EMC) of F. vesca used as strawberry virus indicators apparently carry latent A virus.

Latent A is now widespread in the strawberry stocks in the United States and various strains have been reported to be present in F. vesca and commercial varieties (4). Little is known concerning the relationship of latent A and other viruses in the strawberry or of latent A when present alone in a strawberry plant. Since the virus is detected by an indirect method in the presence of a second virus such as mottle, it would be advantageous if a host could be found that was specific in its reaction to latent A.

A number of years ago at the University of Minnesota, a study of the wild strawberries growing in Minnesota was started to determine if viruses were present and to ascertain their value as indicator hosts for known strawberry viruses. More than 175 collections have been made and tested without results. However, in 1959, a clone of Fragaria virginiana was collected in northern Minnesota (Itasca Co.) which when grafted to a clone of F. vesca[3] developed virus symptoms.

MATERIALS AND METHODS

Indexing Host: The wild strawberry, F. virginiana, used for indexing in this study, was collected in 1959 in northern Minnesota (Itasca Co.). A clone of the collection was established and maintained in a greenhouse. Plants were selected for grafting when one or two stolons were of sufficient size to be easily handled. Whenever possible stolon to stolon grafts were made between the plant to be indexed and the indicator plant using the interlocking tongue system. Some varieties did not produce stolons of adequate number and size in the greenhouse. These plants were jointed to the indicator plants by stolon to petiole grafts in the manner described by Skiles (6).

Grafted plants were then placed on a bench in the greenhouse and examined at regular intervals.

Sources of Virus Isolates: In April of 1960, a number of clones of F. vesca and several varieties infected with different strains of latent A were obtained from Dr. John R. McGrew, Agricultural Research Service, Beltsville, Maryland. In addition, a clone of F. vesca infected with a severe strain of latent A had been obtained from Mr. Spangelo at Ottawa, Canada a number of years ago. The various hosts, virus involved, and sources are as follows:

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[1] Paper No. 4930, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

[2] Professor and Research Assistant, respectively, Department of Plant Pathology and Botany, University of Minnesota, St. Paul.

[3] Shown to be affected with a severe strain of latent A by Dr. John R. McGrew of the Agricultural Research Service at Beltsville, Maryland.

Variety or species[a]	Virus	Source
F. vesca	Severe strain latent A (Sel. 1)	Spangelo, Ottawa, Canada
F. vesca	Severe strain latent A (Sel. 2)	McGrew, A. R. S., Beltsville, Maryland
F. vesca (MEM)	Mild strain latent A	do.
F. vesca (EMK)	Virus free	do.
Blakemore 79-49	Severe strain latent A	do.
Catskill	do.	do.
H-17 (Howard 17) 179-49	do.	do.
Dixieland	do.	do.
Sparkle A	Mild strain latent A	do.
Blakemore 54-20	do.	do.
Florida 90	Virus free	do.
Armore	do.	do.

[a] The plants of these clones of *F. vesca* and varieties had previously been determined to be virus free or infected by Dr. John F. McGrew of the Agricultural Research Service, Beltsville, Maryland.

EXPERIMENTAL RESULTS

The Detection of Latent A on Fragaria virginiana: The production of virus-like symptoms on a plant of a clone of *F. virginiana* when grafted to a *F. vesca* plant (Minnesota clone) containing severe latent A virus led to a series of experiments to determine whether other plants of the *F. virginiana* clone would develop symptoms when grafted to the Minnesota clone of *F. vesca* that was infected with a severe strain of latent A.

Runner grafts were made using the interlocking tongue system of grafting. Grafted plants were placed in the greenhouse and examined at regular intervals.

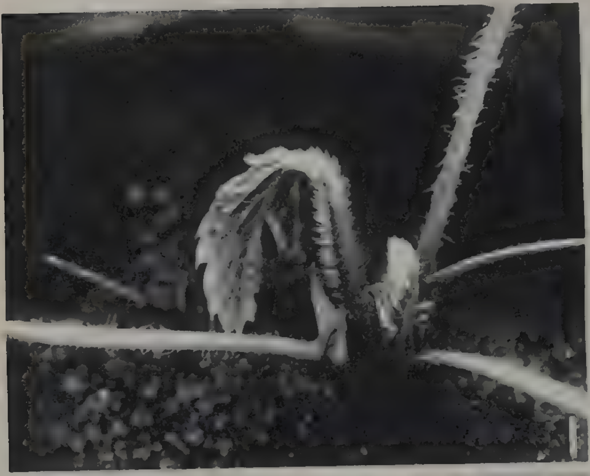
Symptoms began to appear on the *F. virginiana* plants from 2 to four weeks after grafting. The most typical and distinguishing symptoms that developed included:

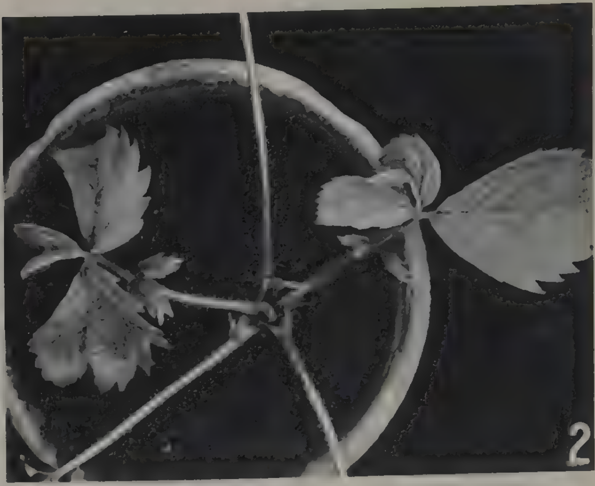
1. Epinasty - This symptom is characterized by recurving of the leaflet midveins, petioles, or both. Two general types of stages of epinasty developed on the infected plants. One type was usually the first symptom on infected plants. It consisted of recurvature of the petiole of newly formed leaflets (Fig. 1). As the leaflets developed the petioles straightened out and grew normally. The other type of epinasty usually occurred on older leaflets and involved the leaflet midveins (Fig. 2). This type of epinasty was often associated with necrosis.
2. Necrosis - This symptom was very conspicuous in all plants infected with the latent A virus. It varied in severity from complete destruction of leaflets (Fig. 3) to small areas of necrotic vein tissue (Fig. 4). Necrotic symptoms were found in all stages of development but did not occur on all leaves.
3. Dwarfing - The degree of dwarfing or stunting varied with the stage of development of virus symptoms. Early stages of development were characterized by extremely shortened petioles and leaflets drastically reduced in size (Fig. 5). Leaves produced later were not so severely stunted.
4. Yellowing and chlorosis - This was especially noticeable on severely dwarfed leaflets (Fig. 5). The degree of yellowing and chlorosis varied greatly with infected leaves. It sometimes resulted in complete chlorosis and premature death of the leaf.

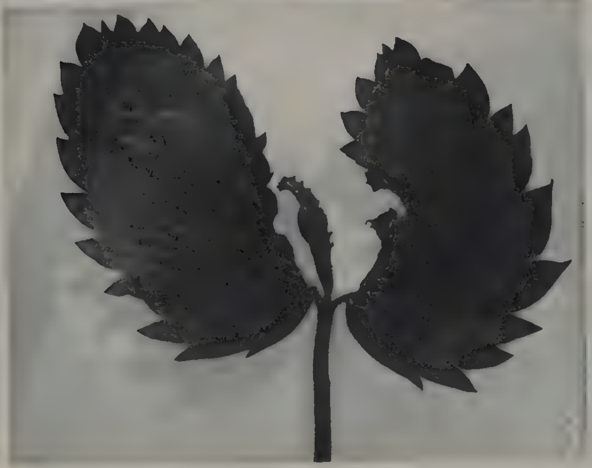
Symptoms occurring on *F. virginiana* were separated into two distinct stages. A definite shock stage occurred with the development of initial virus symptoms. Severe epinasty, dwarfing, and necrosis were apparent. Plants often remained severely stunted and even dormant for short periods of time before producing young leaves characteristic of the recovery stage.

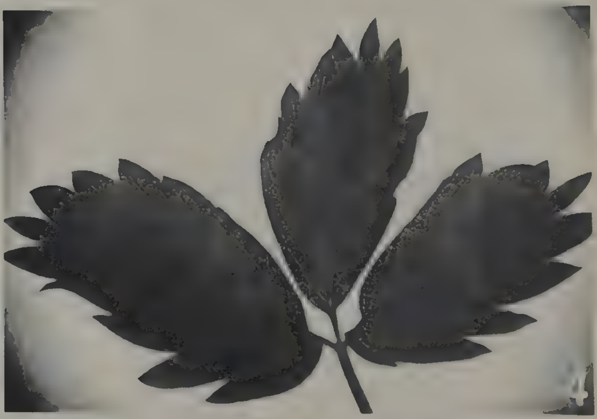
Frazier, in describing latent A (2) and again when describing its effect on the symptoms produced by vein banding virus (3), mentioned this initial shock reaction. All of the *F. virginiana* plants grafted to latent A-infected *F. vesca* plants developed this shock reaction.

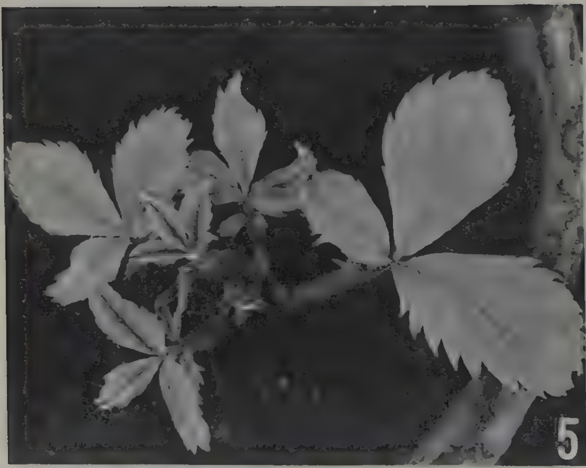
The recovery stage (Fig. 6) began with the formation of new leaves which were not epinastic. These leaves were often discolored brown to purple. The leaves were stunted (Fig. 7)











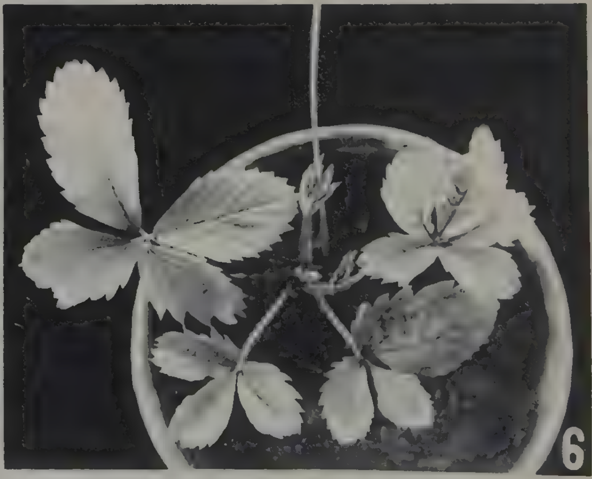


FIGURE 1. An epinasty symptom consisting of the recurvature of the petiole of newly formed leaflets.

FIGURE 2. An epinasty symptom expressed on older leaflets and involving the leaflet midveins.

FIGURE 3. Severe veinal necrosis resulting in complete destruction of the terminal leaflet and distortion of the two other leaflets.

FIGURE 4. Mild veinal necrosis associated with small areas of chlorotic tissue.

FIGURE 5. Dwarfing as expressed on Fragaria virginiana. Note the yellow chlorosis and mild epinasty of the dwarfed leaflets.

FIGURE 6. Leaves showing necrosis, chlorosis and the production of more than three leaflets per leaf.

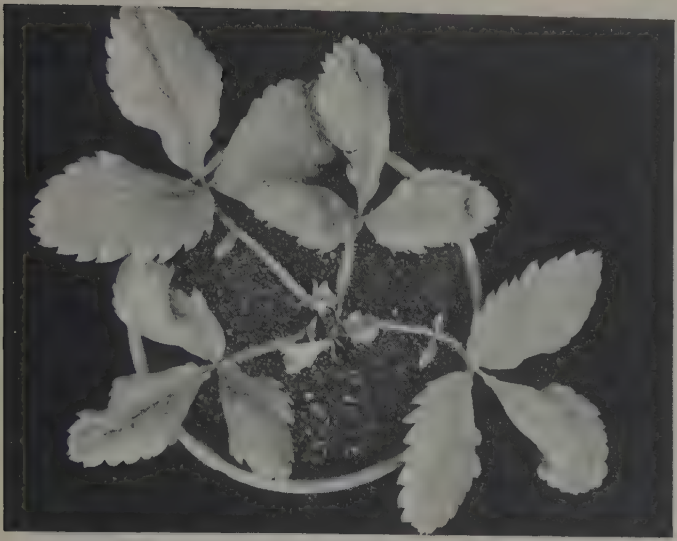


FIGURE 7. Symptoms produced on fully developed leaves caused by latent A virus (severe strain).

but to a lesser degree than in the shock stage. After several stunted but otherwise normal leaves had been produced, other leaves developed showing necrosis, chlorosis, and more than three leaflets per leaf developed. These leaves did not undergo initial epinasty of the petiole. They did show leaflet epinasty which was often accompanied or caused, or both, by necrosis or chlorosis.

An infected F. virginiana plant has been observed for over 1 year. This plant is about half the normal size of an uninfected plant. The crown has proliferated and does not produce runners. About one-fourth to one-third of the leaves show mild necrosis, chlorosis, and leaflet epinasty; the rest of the leaves appear to be normal.

This clone of F. virginiana unquestionably is sensitive to the latent A virus. This virus-host relationship differs from that usually found in F. vesca in that mature, fully developed leaves become twisted and curled and usually die prematurely. This usually occurs 7 to 10 days after initial symptoms are observed.

Effect of Other Sources of Latent A on F. virginiana: Experiments were made to determine whether the clone of F. virginiana could be used to detect other sources of latent A from other sources of F. vesca plants and from commercial varieties. It was also hoped to determine whether various strains of latent A were present and whether they could be detected separately on F. virginiana. Studies using the method of grafting latent A in combination with mild mottle or vein banding virus on F. vesca indicated that at least two strains of latent A were present in the infected donor plants. Table 1 shows the virus condition of the various clones of F. vesca and commercial varieties as determined by the preceding method in Minnesota and previously by McGrew. Also shown are the results obtained when they were grafted to F. virginiana.

F. vesca Selections No. 1 and No. 2 both had severe strains of latent A but they differed in their reaction on F. virginiana. The initial shock reaction of Selection No. 2 was so severe that three of the five indicator plants were killed immediately. In these plants, epinasty, stunting and necrosis occurred on several young leaves before death. In Selection No. 1, a short dormant period occurred but none of the plants was killed as a result of the initial shock reaction.

F. vesca (MEM) caused a mild reaction in F. virginiana. The first symptoms of epinasty, mild vein necrosis and dwarfing occurred about 3 to 4 weeks after grafting. Vein necrosis that caused the leaflets to be slightly crinkled and irregular seemed to be the characteristic symptom of this isolate of latent A. Eight to 10 weeks after grafting the leaflets of the indicator plants were only slightly dwarfed and irregular when compared with a healthy F. virginiana plant.

F. vesca (EMK) did not cause a reaction in F. virginiana and apparently is virus free as was found by McGrew.

The commercial varieties Blakemore 79-49, Catskill, H-17 (Howard 17) 179-49 and Dixieland were all affected with a severe strain of latent A when grafted to F. virginiana. Sparkle

Table 1. The efficiency of Fragaria virginiana as an indicator host for determining the presence of latent A virus in strawberry[a].

Variety or species	Reaction determined by McGrew	Combination reaction[d]	Reaction on Fragaria virginiana
F. vesca (Sel. No. 1)[b]	S	S	S
F. vesca (Sel. No. 2)[c]	S	S	S
F. vesca (MEM)	M	M	M
F. vesca (EMK)	VF	VF	VF
Blakemore 79-49	S	S	S
Catskill	S	S	S
H-17 (Howard 17) 179-49	S	S	S
Dixieland	S	S	S
Sparkle A	M	M	M
Blakemore 54-20	M	M	S & M
Florida 90	VF	VF	VF
Armore	VF	VF	M

[a] S = Severe strain; M = Mild strain; VF = Virus free.
[b] Minnesota collection of East Malling clone of F. vesca.
[c] Collection of East Malling clone of F. vesca obtained from Dr. John R. McGrew, Agricultural Research Service, Beltsville, Maryland.
[d] Reaction determined at Minnesota by grafting to F. vesca in combination with an isolate of the mild mottle virus.

A caused a mild reaction and three of four plants of Blakemore 54-20 were affected with a mild strain of latent A similar to the strain found in the MEM clone of F. vesca. One plant of Blakemore 54-20 caused a severe reaction in F. virginiana. Florida 90 was virus free on F. virginiana and Armore had a mild strain of latent A. There was mild dwarfing; deformed leaflets and veinal necrosis occurred in the initial shock reaction.

The clone of F. virginiana collected in northern Minnesota evidently is an efficient indicator host for determining the presence of the latent A virus in strawberry. This host is sufficiently sensitive to be useful in determining strains of latent A and is capable of indicating the presence of very mild strains. This was demonstrated in the case of the variety Armore which was thought to be virus free.

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ST. PAUL, MINNESOTA

OOSPORE FORMATION OF APHANOMYCES EUTEICHES IN ROOT TIPS OF PISUM SATIVUM AS A METHOD OF EVALUATING RESISTANCE[1]

Thomas H. King and Yong Sup Cho[2]

Summary

Fewer numbers of oospores of *Aphanomyces euteiches* were formed in excised root tips of the resistant Minnesota breeding lines 494 A-1 and 494 A-9 of *Pisum sativum* than in the root tips of the susceptible varieties Perfected Wales and New Era. An intermediate number formed in the moderately resistant Alaska variety. Based on numbers of oospores formed, isolate NY 4 of *A. euteiches* was the most pathogenic; isolate 125 was the next most pathogenic isolate except on the Alaska variety and Minnesota breeding line 494 A-1; isolate 572 was next and isolate 304 the least pathogenic. These results confirm the results of previous studies regarding the relative susceptibility of the varieties of *Pisum sativum* and the Minnesota breeding lines 494 A-1 and 494 A-9 and the relative pathogenicity of the four isolates of *A. euteiches* used.

Determination of the number of oospores that form in excised root tips of pea plants may be an accurate and rapid method of evaluating the resistance or susceptibility of varieties and breeding lines of *Pisum sativum*.

Cunningham and Hagedorn (2, 3) reported that the germ tubes of zoospores of *Aphanomyces euteiches* Drechs. penetrated the root through the root hairs or between epidermal cells and that the zoospores of *A. euteiches* were attracted to the region of the root tip just behind the root cap.

Cho and King (1) in studies on factors affecting infection and oospore formation of *A. euteiches* in excised root tips of *Pisum sativum* showed that the greatest oospore development occurred in the region of the root tip below the region of root hair development and also that infection and oospore development was best in root tips from plants 7 days old. A difference was also observed in the number of oospores that were formed in the excised root tips taken from plants of different commercial varieties and breeding lines. Therefore, studies were made to determine if differences in oospore formation in root tips taken from plants of different varieties could be used as a measure of resistance or susceptibility.

MATERIALS AND METHODS

Three of the four isolates of *A. euteiches* used in these experiments (isolates 125, 304 and 572) were originally obtained from diseased pea plants growing in Minnesota and have been maintained in the Department of Plant Pathology and Botany since 1951. Isolate NY 4 was obtained from Dr. W. T. Schroeder, New York Experiment Station, Geneva, New York.

Seed of the pea varieties New Era, Alaska and Perfected Wales were obtained from the Green Giant Company, Le Sueur, Minnesota. The Minnesota selections 494 A-1 and 494 A-9 have resulted from the project in the Department of Plant Pathology and Botany on the development of lines of *Pisum sativum* resistant to common root rot.

The procedure for obtaining root tips for inoculation was that used by Cho and King (1). Seed was surface sterilized with 1% sodium hypochlorite for 1 minute and allowed to germinate on water agar. After 7 days, root tips measuring 3 to 5 mm in length were removed from lateral roots of plants free of microorganisms. The excised root tips were placed in a zoospore suspension adjusted to a concentration of 30,000 per ml and incubated at 25° C. As checks, additional excised root tips were placed in sterile tap water without zoospores.

Zoospores for inoculum were obtained according to the procedure developed by Schneider and Johnson (4). The fungus was grown on a corn-water medium for 7 days at 20° C after which the mycelial mats were removed, washed in sterile tap water and incubated at 20° for 24 hours. A hemocytometer was used to determine concentration of zoospores.

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[1] Paper No. 4931, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

[2] Professor and Graduate Student, respectively, Department of Plant Pathology and Botany, University of Minnesota, St. Paul.

Table 1. Oospore formation in varieties and breeding lines of peas inoculated with four different isolates of *A. euteiches* and incubated at 25° C.

Plant lines	Incubation period at 25° C	Isolate			
		NY 4	125	572	304
Perfected Wales	24	206	133	145	0[a]
	48	218	174	154	12
	72	219	172	157	12
	96	217	174	157	12
New Era	24	134	126	140	0
	48	160	155	142	0
	72	165	156	146	0
	96	164	155	146	0
Alaska	24	155	81	101	0
	48	163	94	128	0
	72	164	90	120	0
	96	164	91	126	0
494 A-1	24	53	19	53	0
	48	55	21	63	0
	72	57	21	63	0
	96	57	21	63	0
494 A-9	24	51	65	54	0
	48	54	69	68	0
	72	56	68	68	0
	96	56	69	68	0

[a] Average numbers from three replications.

The observations on oospore formation were made 5 days after inoculation.

EXPERIMENTAL RESULTS

Cho and King (1) demonstrated that isolate NY 4 formed more oospores in root tips from plants 7 days old than in root tips from plants 15 and 23 days old. Therefore, root tips were taken from plants 7 days old of the varieties Perfected Wales, New Era, Alaska and from Minnesota lines 494 A-1 and 494 A-9 and inoculated according to the procedures indicated in the section on Materials and Methods. Isolates NY 4, 125, 304, and 572 of *A. euteiches* were used in these studies.

More oospores formed in root tips of commercial varieties of peas, especially Perfected Wales and New Era (Table 1). The least number of oospores were formed in the resistant Minnesota breeding lines 494 A-1 and 494 A-9. In the Alaska variety, oospore formation was intermediate for isolates 125 and 572 but there was little difference between New Era and Alaska varieties in the case of isolate NY 4. Isolate 304, based on previous studies, is weakly pathogenic, and only a few oospores were formed in the root tips of the Perfected Wales variety. In the root tips of the two most susceptible varieties, Perfected Wales and New Era, isolate NY 4 formed the most oospores, then isolate 125, followed by isolate 572 and 304. In the root tips of the moderately resistant Alaska variety and the resistant Minnesota line 494 A-1, isolate 125 formed fewer oospores than NY 4 or 572. Figures 1 and 2 illustrate the differences in oospore formation of isolate NY 4 in the root tips of the susceptible New Era variety and the resistant Minnesota breeding line 494 A-1.

The numbers of oospores that form in excised root tips of pea plants may indicate the resistance or susceptibility of various varieties and breeding lines to *A. euteiches*.

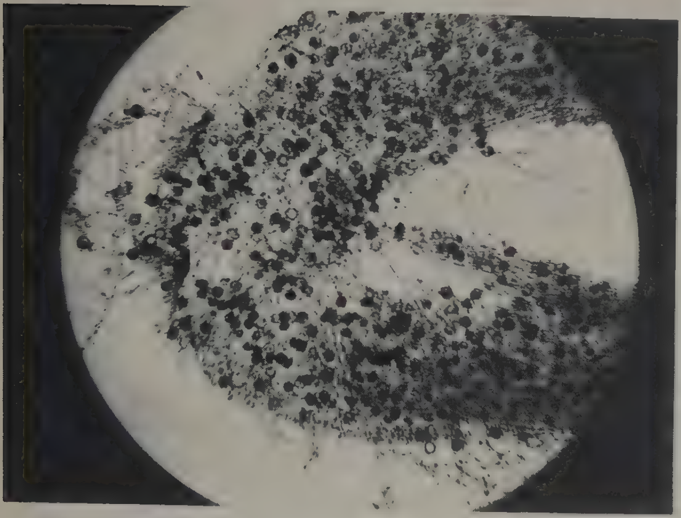

FIGURE 1. (Left) Oospore formation in an excised root tip of a 7-day-old New Era plant inoculated with NY 4 and incubated for 96 hours at 25° C.

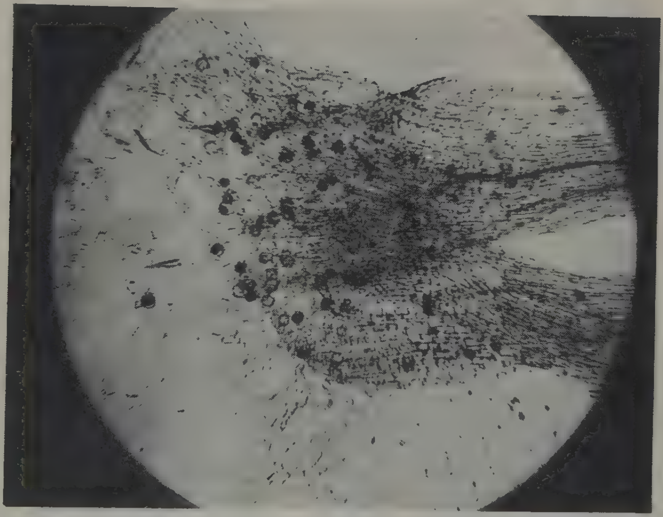

FIGURE 2. (Right) Oospore formation in an excised root tip of a 7-day-old Minnesota-breeding line 494 A-1 plant inoculated with NY 4 and incubated for 96 hours at 25° C.

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NEW HOSTS AND CHARACTERISTICS OF THE PANICUM MOSAIC VIRUS[1]

W. H. Sill, Jr. and Luciano T. Talens[2]

Summary

Four new hosts of Panicum mosaic virus are reported and the symptoms briefly described. The virus has remained infective in desiccated tissue 9 years. Accumulating evidence indicates that this virus is probably endemic in the Great Plains States.

The virus of switchgrass, *Panicum virgatum*, given the name Panicum mosaic virus (PMV) by Sill and Pickett (4) was first seen in 1953 in a grass breeding nursery at the Kansas Agricultural Experiment Station. Investigations by Sill (2), Sill and Desai (3), and Sill and Pickett (4) established the nature and importance of this disease which occurs naturally on *P. virgatum*.

This study was initiated primarily to find a satisfactory local lesion assay host for the virus. Although this purpose was not accomplished, several new hosts were found and are reported.

MATERIALS AND METHODS

All plants tested were started from seeds in Vermiculite. When the seedlings were 3 to 5 inches high, they were transplanted into potting soil and grown in flats. Hyponex fertilizer in solid form or in aqueous suspension was added as needed to assure vigorous growth.

Plants were grown in a greenhouse provided with an evaporative cooler in summer. Summer temperatures varied from 60° to 95° F and winter temperatures were held at 72° ±3°. Insects were controlled by weekly fumigation with Plantfume 103. In the summer the insecticide malathion and the miticide Kelthane were also utilized as sprays.

All plants were inoculated by the routine carborundum rubbing method. The inoculum was diluted 1:10 in tap water and was extracted from frozen infected *Digitaria sanguinalis* leaf tissue. Healthy control plants were usually grown beside treated plants but were given no treatment.

EXPERIMENTAL RESULTS

PMV was transmitted mechanically to five already known susceptible hosts (4) and to 4 other related grass species (Tables 1 and 2).

Setaria sphacelata was immune and *Panicum maximum* reported (4) previously to be immune was immune in the present trials also. *Panicum antidotale* did not develop symptoms, but when extracts from inoculated plants were used to inoculate *D. sanguinalis* two of six plants developed symptoms, indicating that *P. antidotale* may be a symptomless carrier.

Several unrelated plants were also immune from PMV. These plants were: *Phaseolus aureus*, *P. acontifolius*, *P. calcaratus*, *Vigna sinensis* (var. Black), *Zinnia elegans*, *Piper nigrum*, *Zea mays*, *Datura stramonium*, *Cucumis sativus*, and *Cucurbita pepo*. Earlier studies (2, 3, 4) showed many other plants to be immune. All tests would indicate that PMV has a narrow host range within the grass family.

Symptoms on the newly reported host plants (Table 2) were as follows:

Panicum ramosum: Primary symptoms were faint chlorotic, longitudinal, discrete streaks or specks. These gradually enlarged to become a severe yellow chlorosis, then necrosis, and finally some of the plants died (Fig. 1). The incubation period was about 7 days at summer temperatures.

Panicum decompositum: Early symptoms were a faint chlorosis. Irregular patches sometimes were more prominent at the base of the leaf. These patches gradually increased in size and numbers, giving a distinct green-yellow mottle followed by necrosis. A very severe necrosis developed rapidly, and plants usually lived about 12 days after symptoms appeared. The incu-

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[1] Contribution No. 605, Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, Manhattan. Botany serial No. 767.

The authors wish to express their appreciation to the following men who furnished seed for the experiments: Kling L. Anderson, L. C. Newell and Willis H. Skrdla.

[2] Associate Professor and Graduate Research Assistant, Department of Botany and Plant Pathology, Kansas State University.

Table 1. Grass species previously reported susceptible to Panicum mosaic virus and confirmed in these experiments.

Host	Number of trials	Ratio of plants infected to no. of plants inoculated		Reaction[a]
		Sill and Pickett	Present experiments	
Panicum miliaceum	2	8/12	46/46 10/14[b]	L
Setaria italica	3	8/22	104/106	M-S
Setaria lutescens	1	4/19	+[c]	M-S
Panicum virgatum var. Blackwell	1	8/20	+	M-S
Digitaria sanguinalis	1	51/62	+	M-S

[a] L = systemic lethal; M = mild mottle; S = severe mottle.
[b] Age of plant when inoculated was at fruiting stage but virus was still lethal.
[c] Positive symptoms but quantitative data not recorded.

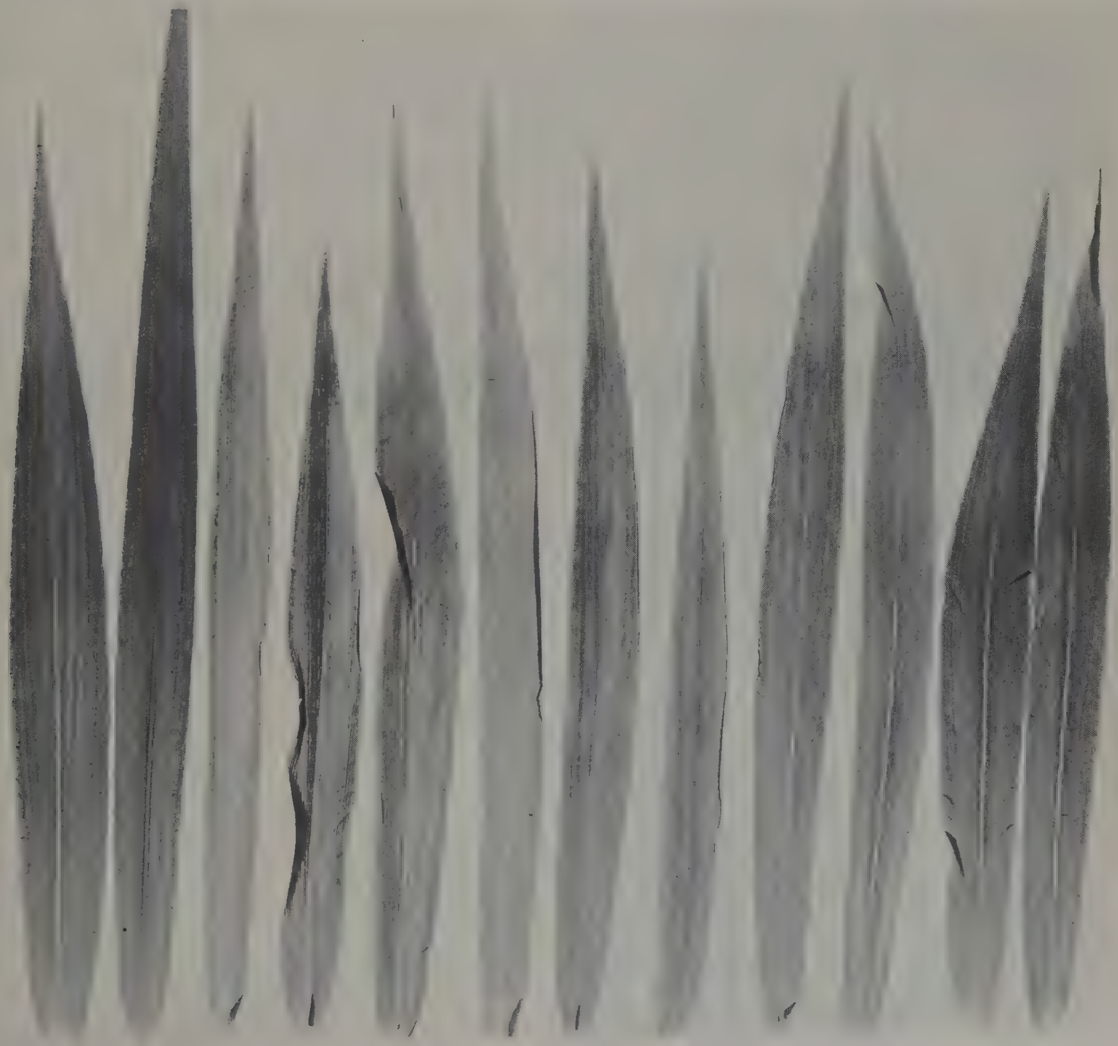

FIGURE 1. Symptoms of Panicum mosaic virus on *Panicum ramosum*. Healthy leaf at left.

Table 2. New grass species found susceptible to Panicum mosaic virus.

Host	Number of trials	Ratio of plants infected to no. of plants inoculated	Reaction[a]
Panicum turgidum	2	64/71	M-S
Panicum ramosum	1	47/64	S-L
Panicum decompositum	1	27/27	L
Setaria verticillata	2	52/52	M
		23/29[b]	M

[a] M = mild mottle; S = severe mottle; L = systemic lethal.

[b] Inoculations made at fruiting stage.

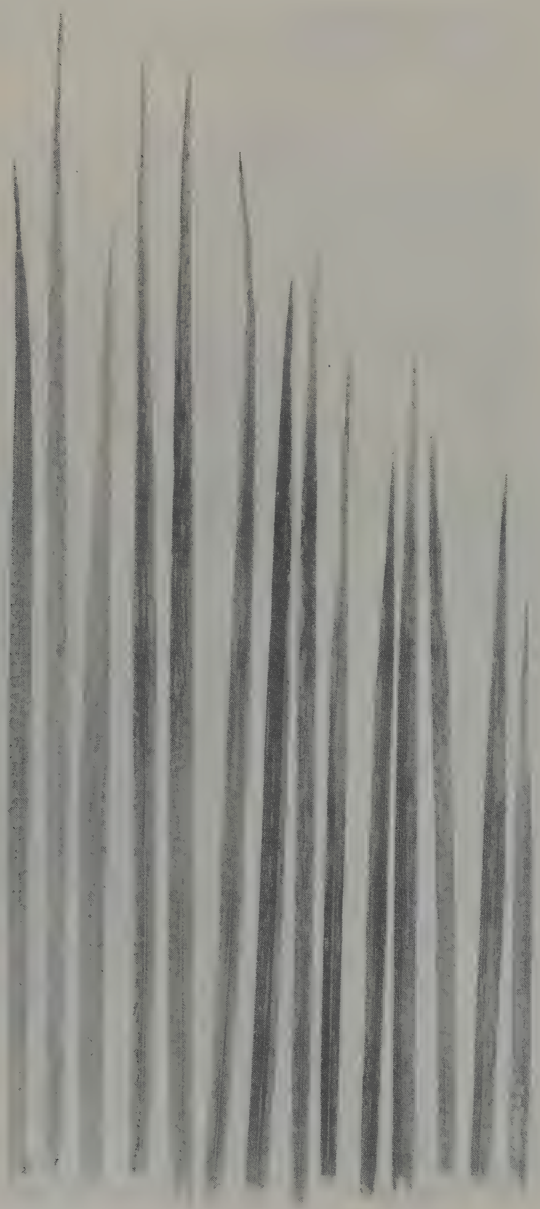

FIGURE 2. Symptoms of Panicum mosaic virus on *Panicum turgidum.* Healthy leaf at left.

bation period was about 4 days at summer temperatures.

Panicum turgidum: Discrete diffuse yellow spots or streaks were the primary symptoms. These spots or streaks enlarged until they finally coalesced and gave the typical yellow-green mottle pattern on infected plants. Plants were not severely stunted even though severe mottling was observed (Fig. 2). The incubation period was 12 to 18 days during summer months.

Setaria verticillata: Symptoms first appeared as very faint chlorotic spots and streaks mostly on young lateral shoots. Mature inoculated leaves often did not show symptoms until plants had been infected for several weeks; then a mild mottle developed. Infected plants produced seed normally. Initial symptoms appeared in approximately 9 days at summer temperatures.

PMV has remained infective in desiccated leaf tissue for 9 years. However, the infectivity of tissue stored in 1953 by the method of McKinney (1) is considerably reduced and it appears doubtful that this tissue will remain infective much longer.

Incubation periods varied in different hosts at varying temperatures in this as well as in previous studies (2, 4), but generally were shorter at the more ideal warm temperatures and longer at cooler temperatures. Without question PMV is a warm temperature virus. Optimum symptoms developed on many hosts at temperatures as high as 85° to 95° F. No masking from high temperatures was apparent at any time. This evidence along with the fact that PMV occurs naturally in an important native perennial grass, P. virgatum (4), suggests that it may be an endemic virus in the high Great Plains States since high summer temperatures are characteristic of this area.

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DOWNY MILDEW OF PEAS PREVALENT IN PACIFIC NORTHWEST IN 1962

R. E. Ford and M. J. Silbernagel[1]

Downy mildew of peas (*Peronospora viciae* (Berk.) d By. (*P. pisi* Syd.)) was fairly widespread in the pea-growing areas of the Pacific Northwest in 1962. The frequency ranged from only a trace in some areas up to 100% of the plants infected in others. This occurrence is considered noteworthy, because downy mildew is usually not observed in the Northwest outside of the Puget Sound area of northwestern Washington, where both local and systemic infections occur uniformly each year without serious damage. Downy mildew did not appear to be damaging except in a few early-planted fields in conjunction with hot, dry winds.

Factors responsible for the occurrence of downy mildew outside its usual range were unseasonably cool and rainy weather during a 6-week period from late April until early June throughout the Pacific Northwest. The monthly weather summaries for Washington, Oregon, and Idaho show the monthly average to be 5° to 6° F below normal for May in the pea-growing areas. Rainfall, although only slightly greater than normal, was so frequent that relative humidity was high for long periods during the 6 weeks. This cool, damp weather induced heavy succulent vine growth and allowed ample time for spore dissemination and host infection. The rainfall was not heavy enough to increase soil moisture sufficiently to compensate for the much heavier than normal vine growth.

Two commercial seed-increase fields near Pullman, Washington that had been planted 3 to 4 weeks before the normal planting time for the area were the only fields observed to have suffered any appreciable damage. They contained large, unevenly maturing, grey, irregular areas involving about 50% of the plants. In these irregular areas the plants were dead after about a week of warm, dry winds, and all plants inspected were infected with downy mildew. A major debilitating factor seemed to be the lack of adequate soil moisture to carry the large vine plants completely to maturity, but the heavy infection by the downy mildew fungus must have exerted a measurable water stress on the plants. Therefore, many of the youngest pods failed to fill on the infected vines. A quick transition from cool, damp weather to warm, dry weather did not allow much time for "hardening-off" of the plants. In spite of this, however, slightly higher than average seed yields were obtained from these fields, indicating that the advantage gained from early planting was still greater than the reduction in yield caused by downy mildew and the apparent lack of moisture. The progress of downy mildew appeared to be halted by the warm, dry weather of mid-June. Downy mildew is very rare in eastern Washington.

Downy mildew was also present but apparently not damaging in the Nezperce, Idaho area and the Palouse-Blue Mountain pea-growing region of Washington, but no fields or test plot contained more than 1 to 5% infected plants. Only traces of downy mildew were found in the pea seed-production area of Twin Falls, Idaho, but its occurrence is noteworthy since this area is typically warm, dry, and free from downy mildew.

No downy mildew was observed in the Columbia Basin in Washington or in the Willamette Valley in Oregon, although cool, rainy weather prevailed.

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INVASION OF AND DAMAGE TO BEAN SEED BY STORAGE FUNGI[1]

Luis Cesar Lopez F. and C. M. Christensen[2]

Summary

Seeds of five varieties of beans from Mexico and three from the United States were stored at relative humidities of 76, 80, and 85% at temperatures of 20° to 25°C and at intervals of 2 months tested for moisture content, germination, and number and kinds of storage fungi. Equilibrium moisture contents were about 16% at 76% r.h., 17% at 80% r.h., and 19% at 85% r.h. Storage fungi, mainly of the *Aspergillus glaucus* group, and particularly *A. restrictus*, increased and germination percentage of the seeds decreased with increasing time and increasing moisture content. Abnormal and crippled seedlings such as "baldhead" and "snakehead" evidently resulted from invasion of the seeds by storage fungi. Invasion occurred through the hilum and micropyle as well as through cracks in the seed coat, and the embryo root, stem and growing point appeared to be attacked in preference to the cotyledons.

INTRODUCTION

Tests by the senior author in 1960 of a number of samples from different lots of beans, *Phaseolus vulgaris*, that had been stored at the Cotaxtla Experiment Station near Veracruz, Ver., Mexico, and the germination of which had decreased greatly during storage, showed that they were heavily invaded by storage fungi, and particularly by *Aspergillus restrictus* Smith. Invasion by this species and by other species of *Aspergillus* has been shown to reduce the germination percentage of wheat (2, 7, 10), barley (9), corn (8), and peas (5), but no information is available concerning the conditions that permit invasion of beans by these fungi, the means of invasion, or the effect of such invasion on the germination of the seeds or vigor of the seedlings. The work reported here was undertaken to provide such information.

MATERIALS AND METHODS

Beans Tested: Three varieties of beans grown in the United States, Black Eye, Michigan Pea, and Pinto, were obtained from a store in St. Paul. All germinated 88 to 92%; when surface disinfected and cultured on malt-salt agar, *A. glaucus* Link grew from 20% of the seeds of the Michigan Pea variety, but no storage or other fungi grew from any of the others. Seeds of five varieties were obtained from the Agricultural Experiment Station at Chapingo, Mexico: Amarillo 153, Bayomex, Canario 101, Jamapa, and Negro 150. These were from carefully harvested seed stocks, all had a moisture content of 9 to 10% when received, and germinated 98 to 100%. *A. ruber* (Spieck. & Brem.) T. & C. grew from 10% of surface disinfected seeds of the variety of Negro 150 cultured on malt-salt agar, but storage fungi grew from less than 2% of the seeds of other varieties so cultured.

Storage: Quantities of 300-500 grams of each variety were inoculated with dry spores of *A. restrictus*, *A. repens* (Cda.) d By., *A. amstelodami* (Mangain) T. & C., and *A. ruber* (all in the *A. glaucus* group species), *A. candidus* Link, *A. ochraceus* Wilh., *A. flavus* Link, and *Penicillium* sp. The seeds of each variety were then put in an open pint jar or in a cheesecloth bag, and these placed in desiccators containing saturated solutions of sodium chloride, ammonium sulfate, and potassium chloride, to maintain relative humidities of 76, 80, and 85%, respectively (11). The desiccators were kept at a temperature of 20° to 25°C.

Moisture contents were determined by the 2-stage, air oven method (1) and are expressed on a wet weight basis.

Germination: Fifty or 100 seeds were planted in autoclaved sand in a greenhouse at a temperature of 20° to 25°C, and the seedlings counted after 8 to 14 days.

Percentage of Surface Disinfected Seeds Yielding Fungi: Seeds were shaken for 1 minute in a 1% solution of sodium hypochlorite, rinsed in sterile water, and cultured on malt agar con-

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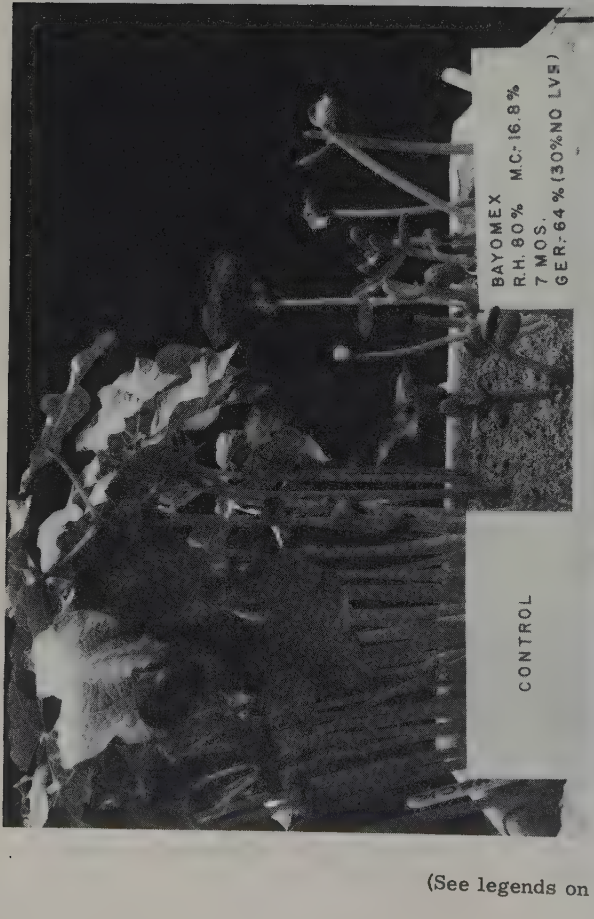
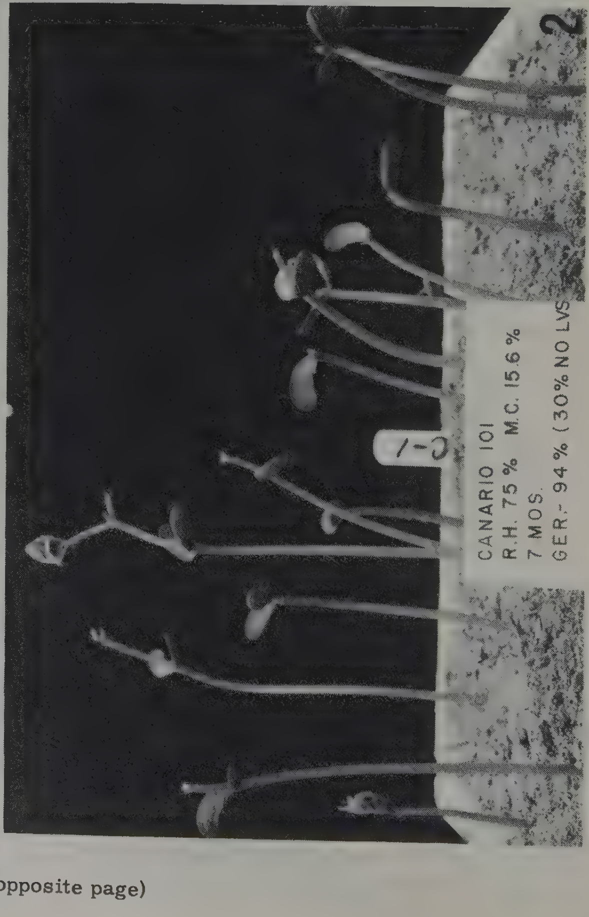
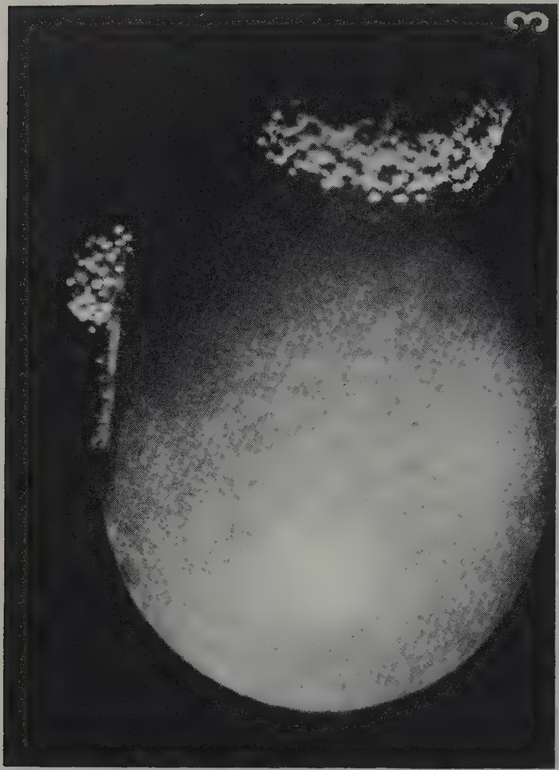
[2] Fellow of The Rockefeller Foundation and Professor of Plant Pathology, respectively, Department of Plant Pathology and Botany, University of Minnesota, St. Paul.


BAYOMEX
R.H. 80% M.C.-16.8%
7 MOS.
GER.- 64% (30% NO LVS)
CONTROL

1


CANARIO 101
R.H. 75% M.C. 15.6%
7 MOS.
GER.- 94% (30% NO LVS
1-C

2



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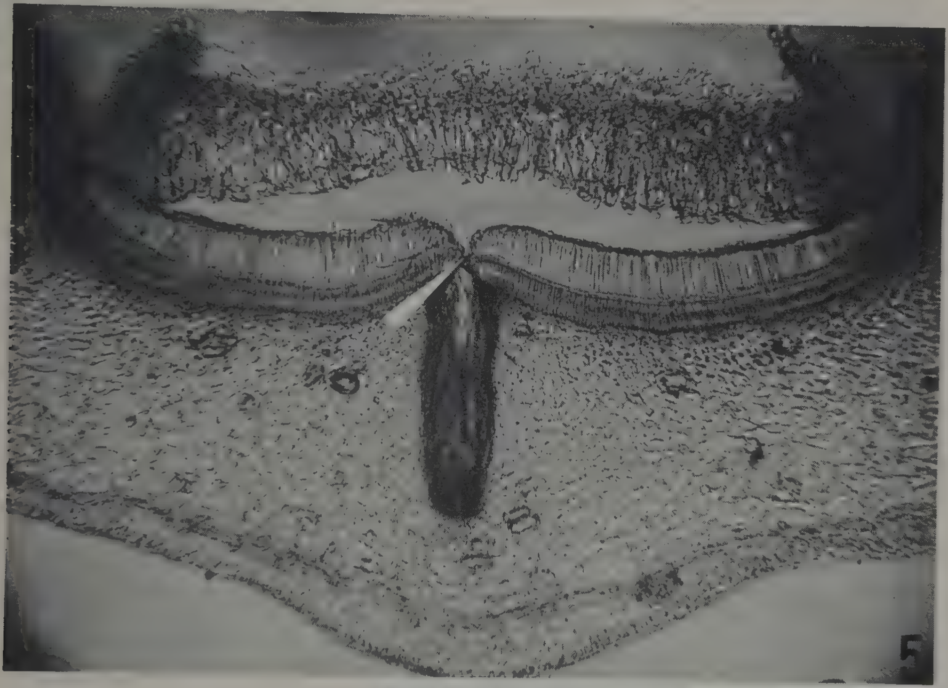
taining 10% sodium chloride. Usually 50 to 100 whole seeds were so cultured, and 50 to 100 seeds were surface disinfected, split aseptically, and placed on malt-salt agar with the split sides up, so that it was possible to see from what portion of the interior of the seeds the fungi grew.

Colonies per Gram of Seed: Five grams of seed were comminuted 1.5 minutes in 500 ml of sterile 0.12% agar solution in a Waring Blendor, 5 ml of the suspension removed and placed in 45 ml of sterile 0.12% agar solution, shaken 100 times (further dilutions, when necessary, were made in the same way) and 1 ml of one or more dilutions put in each of two Petri dishes, malt agar containing 10% sodium chloride, melted and cooled to 50°C added, the dishes swirled to distribute the suspension uniformly, and the dishes kept at room temperatures until the colonies could be counted and identified. Both of the above procedures are now more or less standard for determining the extent to which storage fungi have invaded seeds.



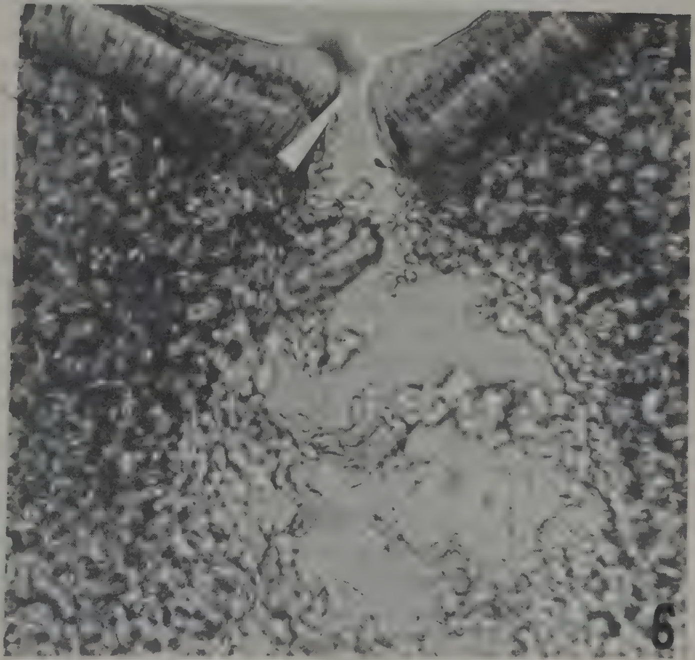
FIGURES 1 and 2. Abnormal seedlings that developed from beans stored under the conditions indicated on the labels.

FIGURE 3. Aspergillus restrictus growing from micropyle and from crack in the seed coat of an infected bean.

FIGURE 4. Aspergillus repens growing out from the interior of a bean through scratches that penetrated through the palisade layer.

FIGURE 5. Section through hilum of the seed coat of a bean; arrow points to the place where fungi might enter.

FIGURE 6. Section through micropyle (arrow) of the seed coat of a bean; in many such sections mycelium was visible in the cavity beneath the micropyle.



RESULTS

The increase in moisture content with increasing time of storage, and the associated increase in storage fungi and decrease in germination percentage of the seeds, are summarized in Table 1. The moisture content of the beans continued to increase up to the end of the test, although the increase from the sixth to the eighth month was very slight, and the final moisture contents probably were very nearly in equilibrium with the different relative humidities. The final moisture contents were about 1% higher than equilibrium moisture contents of wheat and corn stored at the same relative humidities.

Table 1. Influence of moisture content and time on germination and number and kinds of fungi in beans stored at 20° to 25° C.

Months stored	% moisture content (wet weight)	% germination		% surface disinfected kernels yielding		Colonies/gram of grain (in thousands)	
		normal	abnormal	Aspergillus restrictus	Aspergillus repens	Aspergillus restrictus	Aspergillus repens
5 varieties of Mexican beans							
76% relative humidity							
2	14.7	99	0	0	1	0.05	0
4	15.4	80	18	8	11	0.5	0
6	15.8	80	15	17	59	10	3
8	15.8	64	14	38	62	18	18
80% relative humidity							
2	14.7	99	0	0	2	0.4	0
4	15.7	81	14	7	3	4	0
6	16.8	52	30	23	44	44	10
8	17.1	14	35	50	53	73	21
85% relative humidity							
2	17.2	99	0	0	14	2.5	0
4	18.6	73	13	37	42	2334	0
6	18.9	0	0	43	47	1840	215
8	18.9	0	0	46	58	2223	733
3 varieties of U. S. beans							
80% relative humidity							
2	14.1	93	0	0	7		
5	15.5	83	5	1	10		
7	16.5	43	17	12	42		
9	17.3	0	3	28	66		

In general, storage fungi increased and germination percentage of the seeds decreased with increasing moisture content and increasing time of storage. Seeds of the variety Jamapa stored at 76% relative humidity germinated 100% after 8 months; seeds of the variety Bayomex stored under the same conditions germinated only 65%, and 30% of the seedlings were abnormal; seeds of the variety Amarillo germinated 57%, and 15% of the seedlings were abnormal. Seeds of the variety Jamapa had very sound coats, with no detectable breaks or cracks, while many of the seeds of the varieties Bayomex and Amarillo 153 had obvious breaks and cracks in the coats, and these breaks and cracks probably allowed ready invasion by storage fungi.

Types of abnormal seedlings produced by seeds stored under these conditions are illustrated in Figures 1 and 2. Many of the abnormal seedlings closely resembled "baldhead" and "snakehead" that result from mechanical injuries (3, 4, 6). Evidently these abnormalities can also result from storage conditions that permit invasion by storage fungi. In corn and wheat stored at the same relative humidities as those used in the present tests, storage fungi have been proven to be chiefly responsible for the death of the embryos (2, 8). Conclusive evidence on this point with beans can be obtained only by storing beans free from storage fungi and inoculated with storage fungi at the same moisture contents.

Mode of Invasion of Beans by Storage Fungi: When beans that had been invaded by storage fungi were surface disinfected and cultured on malt-salt agar, the fungi grew out through the hilum and micropyle, or through cracks in the seed coat (Fig. 3), but never through the unbroken seed coat. If coats of seeds invaded by storage fungi were punctured, the fungi grew out through these punctures (Fig. 4).

The hilum and micropyle of at least some varieties of beans appear to offer ready entrance to storage fungi. Sections through these structures are shown in Figures 5 and 6. The micropylar opening of seeds of some varieties is relatively large and can be seen easily with a magnification of X10; inward from this opening are thin-walled parenchyma cells through which the fungi probably can grow and reach the embryonic root. Beans with sound seed coats, stored several months at relative humidities of 76 and 80%, were surface disinfected, split aseptically, cultured with the split sides up on malt-salt agar, and observed daily. Aspergillus, especially A. restrictus, grew first from the embryonic root adjacent to the micropyle. With increasing time of storage the fungus grew up into the embryonic stem and into the apical bud. Seeds with cracked or broken seed coats were invaded much more rapidly by storage fungi than those with sound seed coats, and in these seeds the embryonic root, stem, and growing point appeared to be invaded in preference to the primary cotyledons.

Seeds with no visible cracks in the seed coat were stored 6 months at 76% relative humidity, surface disinfected, and the seed coats removed and cut into portions containing the hilum and micropyle and not containing these structures. All the pieces were again surface disinfected briefly, and cultured. A. restrictus grew from 60% of 188 pieces of seed coat containing the hilum and micropyle, and from only 2% of 794 pieces not containing the hilum and micropyle.

Seeds of the variety Jamapa, as mentioned above, had very few cracks in the coat. Seeds of this variety were scratched artificially, just deeply enough to penetrate through the palisade layer of the coat, incubated 4 months at 85% relative humidity, then tested for germination percentage and for number and kinds of fungi. Thirty-five percent of the seeds were dead, 25% produced abnormal seedlings, and 40% produced normal seedlings; dilution cultures yielded 9 million colonies of A. restrictus and 1 million colonies of A. repens per gram of seed. Compared with the same storage period for nonwounded seeds of this variety, wounding resulted in heavier invasion by storage fungi and a somewhat greater decrease in germination percentage.

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LOSSES CAUSED BY RHIZOCTONIA SOLANI BORNE ON BEAN SEED[1]

M. Chorin and A. Halfon-Meiri[2]

Rhizoctonia solani Kuehn has variously been reported to be borne on seeds of Phaseolus vulgaris (1, 2, 3), but the extent of damage caused by such seed infection has not been assessed. Seeds collected from Brittle Wax beans severely affected by this pathogen in the Israel coastal plain in 1960 afforded an opportunity to determine the economic importance of its transmission by seed.

To verify pathogenicity, a R. solani isolate from these bean seeds was cultured on potato-dextrose agar, and sound seeds were inoculated by placing bits of mycelium from these cultures on them. This resulted in 75% infection, with typical symptoms on the seeds and young seedlings.

The effect of the pathogen on viability of seeds and seedlings was determined by germination tests on 10 random samples, each comprising 300 seeds, collected from the affected fields. In seven samples the percentage of abnormal seedlings amounted to 16 to 24, in two samples to 34 and 43, respectively, and only in one sample were all the seedlings normal. The percentage of seeds that failed to germinate amounted in five cases to 4 to 8 (which is probably normal for sound seeds), in two cases to 16 to 20, and in three cases to 50 to 60. In the sample most severely affected only one healthy seedling emerged, while there were 43 abnormal seedlings and 56 seeds failed to germinate.

The seeds were graded for infection by R. solani, according to outward symptoms: in two graded lots of seeds those bearing brown spots comprised 60 to 90% of infected seedlings and 10 to 40% of seeds failing to germinate, so that not a single healthy seedling was produced; seeds bearing yellow spots yielded 10% of healthy and 20 to 40% of abnormal seedlings, while 50 to 70% of these seeds failed to germinate; seeds with no outward symptom produced 90 to 100% of sound seedlings, with 0 to 10% failing to germinate.

The commercial value of seed grading was further shown by germination tests with ungraded lots of seeds and lots consisting only of seeds free from symptoms or only of seeds with brown or yellow spots. The results (Table 1) indicate that failure to grade these seeds resulted in marked reductions of the percentage of germination and healthy seedlings.

Table 1. Results of grading seeds by outward symptoms of infection by Rhizoctonia solani.

	Ungraded seeds		Seeds free from spots		Seeds with brown or yellow spots	
	lot A	lot B	lot A	lot B	lot A	lot B
% total germination	84	80.7	93	93	40	44
% healthy seedlings	60	64.7	93	91	6	1
% infected seedlings	24	16.0	0	2	34	43
% seeds failing to germinate	16	19.3	7	7	60	56

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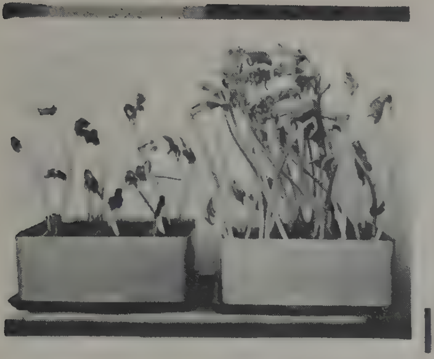

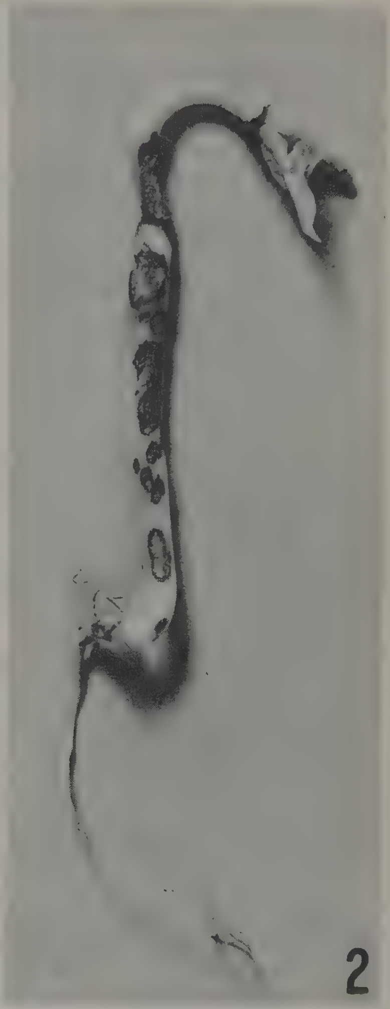

FIGURE 1. Left -- Bean seedlings infected by Rhizoctonia solani. Right -- Healthy seedlings.
FIGURE 2. Bean seedling badly infected and spotted by Rhizoctonia solani.

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AMMONIA INJURY TO APPLES AND PEACHES IN STORAGE[1]

E. Brennan, I. Leone, and R. H. Daines[2]

Summary

Apple and peach varieties were subjected to controlled ammonia fumigations in order to reproduce symptoms encountered on such fruit in storage. Varieties of both fruits were compared as to extent of injury at varied fumigation levels, and their capacities to absorb ammonia as reflected by total nitrogen content. Although Red Rome and Red Delicious apple varieties were particularly sensitive, there was no distinguishable difference among the peach varieties Elberta, Blake, and Red Crest. While chemical analyses did indicate immediate absorption of the gas by the fruit skin, since the elevated concentration was of such short duration, this indication was not considered to be of value in diagnosing accidental fumigation.

INTRODUCTION

Occasionally, upon removal from storage, certain varieties of apples show a characteristic prominence of the lenticels, which can be attributed to the accidental release of ammonia in the refrigeration system of the storage house (3). Peaches held in storage have also been damaged by ammonia fumes, although the expression of injury differs somewhat from that of the apple. Certain peach varieties show discrete spots, others exhibit large brown patches resembling bruises.

MATERIALS AND METHODS

To verify these observations and to investigate other aspects of ammonia absorption by apple and peach, experimental fumigations were conducted in the laboratory. Fumigations were carried out in a 2 x 4 foot wooden chamber. Ammonia was evolved by passing dry air through appropriate concentrations of ammonium hydroxide which was then metered and introduced through glass tubing into the chamber. Depending on the duration of the fumigation period, air samples were withdrawn at 20- to 30-minute intervals into midget impingers containing sulfuric acid and titrated with sodium hydroxide to obtain the existing ammonia concentration. In this way the concentration of the gas was kept constant during the fumigation period. When fruit was removed from the chamber, representative samples were peeled as thinly as possible and the peelings air-dried for total nitrogen analysis by the Kjeldahl method. The fruit was also sampled at 2, 3, and 7 days after fumigation to determine the persistence of any absorbed ammonia. Macroscopic and microscopic observations were made of the injured fruit.

Apple fumigation studies were begun in May 1961 with fruit that had been in storage since the previous October. Newly harvested apples were tested in the fall of 1961, and stored fruit was again evaluated in the spring of 1962. Five varieties were included in the study: Red Rome, Regular Rome, Stayman, Red Delicious, and Golden Delicious. Several rates of fumigation were used but only three will be reported: 0.03% NH_3 for 28 hours, 0.12% for 6 hours, and 0.33% for 2 hours.

Peach fumigations were carried out in September 1961. The varieties studied were Elberta, Blake, and Red Crest. One-hour fumigations were conducted at 0.05, 0.15, 0.33, 0.35% NH_3; and 6-hour fumigations at 0.04 and 0.02%.

RESULTS AND DISCUSSION

That injury induced by ammonia in apple was indeed adjacent to the lenticel was verified by microscopic examination. Exposures to ammonia caused the tissue immediately surrounding the lenticel to become purple or black (Fig. 1, above right), the anthocyanins of the skin acting as an indicator to the alkaline reaction (4). At low concentrations (0.03%) the dark coloration disappeared within a few hours after removal of the apples from the ammonia atmosphere. At higher concentrations (0.12 and 0.33%), though the blackened area lost much of its color, with-

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Table 1. Extent of injury on ammonia fumigated storage and newly harvested apples.

Fumigation rate	Extent of injury							
	Red Rome	Red Delicious		Stayman		Regular Rome	Golden Delicious	
	stored	stored	fresh	stored	fresh	stored	stored	fresh
0.03%/28 hr	T[a]	T	-	T	-	T	-	-
0.12%/6 hr	+++	++	T	++	T	+	-	-
0.33%/2 hr	+++	++	+	+	T	+	-	-

[a] T = Transitory injury, immediate blackening that fades within hours. - No injury. + Slight injury. ++ Moderate injury. +++ Severe injury.

Table 2. Extent of ammonia injury on peaches.

Fumigation rate	Extent of injury		
	Elberta	Blake	Red Crest
0.05%/1 hr	T[a]	T	T
0.15%/1 hr	++	++	++
0.35%/1 hr	+++	+++	+++
0.02%/6 hr	T	T	T
0.05%/6 hr	+	+	+

[a] T = Transitory injury, immediate blackening that fades within hours. + Slight injury. ++ Moderate injury. +++ Severe injury.

Table 3. Total % nitrogen content of peelings of ammonia fumigated storage apples.

Variety	Control	0.03%/28 hr		0.33%/2 hr		
		immed.	2 days	immed.	3 days	7 days
Red Rome	0.38	0.44	0.38	0.50	0.50	0.38
Red Delicious	0.38	0.38	0.32	0.44	0.38	0.38
Regular Rome	0.44	0.44	0.38	0.44	0.44	0.44
Stayman	0.50	0.50	0.50	0.63	0.58	0.58
Golden Delicious	0.38	0.38	0.38	0.50	0.50	0.44

Table 4. Total % nitrogen content of peelings of ammonia fumigated newly harvested apples.

Variety	Control	0.09%/4 hr		0.33%/2 hr		0.88%/3 hr	
		immed.	3 days	immed.	3 days	immed.	3 days
Red Delicious	0.25	0.25	0.25	0.25	0.31	0.49	0.31
Stayman	0.31	0.25	0.25	0.31	0.39	0.66	0.52
Golden Delicious	0.31	0.25	0.25	0.31	0.45	0.66	0.31

in 24 hours the area around the lenticel assumed a brown "corky" appearance giving greater prominence to the lenticels on the fruit (Fig. 1, below). The red side of the fruit was more noticeably affected by the gas than was the yellow side.

Apple varieties showed inherent differences in ammonia susceptibility (Table 1); Red Rome and Red Delicious were the most sensitive, Stayman and Regular Rome were intermediate, and Golden Delicious was least sensitive. Although Paragon was included in only a few of the tests, it appeared to occupy a position similar to Golden Delicious. Golden Delicious apples presented a problem in the evaluation of ammonia damage. As it is grown in New Jersey, this

Table 5. Total % nitrogen content of peelings of ammonia fumigated peaches.

Variety	Control	.05%/1 hr			.15%/1 hr			.35%/1 hr			.02%/6 hr			.05%/6 hr	
		immed.	3 days	7 days	immed.	1 day	2 days	immed.	2 days	5 days	immed.	2 days	5 days	immed.	3 days
Elberta	0.70	0.71	0.77	0.91	1.32	1.77	0.66	1.32	1.54	1.18	0.91	0.78	0.72	0.63	0.62
Blake	0.22	0.85	0.62	0.44	1.73	1.04	0.63	0.98	1.76	1.58	0.82	1.20	1.13	0.63	0.47
Red Crest	0.50	1.10	0.98	0.44	0.50	0.54	0.88	1.51	1.91	1.29	1.23	0.52	0.72	0.52	0.66

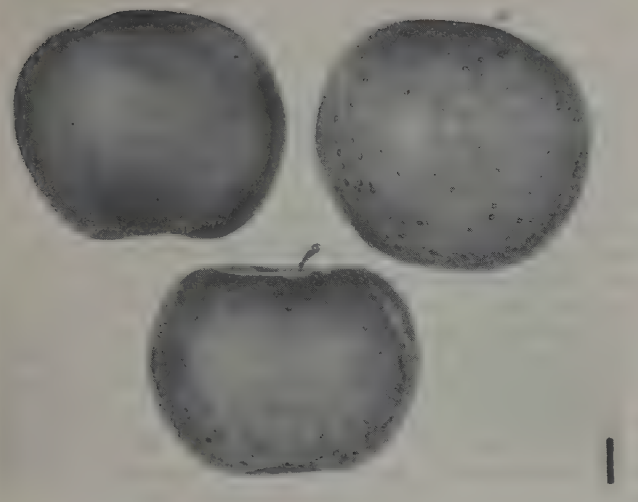
variety characteristically has enlarged "corky" lenticels. After exposure to ammonia there was difficulty in detecting a change in the nature of the lenticels, and therefore the variety was assumed to be tolerant of ammonia. The Golden Delicious variety as grown in the State of Washington has, by comparison, a smooth, unbroken skin with lenticels that are barely visible. Upon fumigation, these apples showed the characteristic injury pattern progressing from a blackened stage to the development of prominent corky lenticels, indicating that Golden Delicious apples are sensitive to ammonia.

Newly harvested apples were more resistant to ammonia injury than were those that had been held many months in storage (Table 1). Whereas 0.33% NH_3 for 2 hours caused considerable injury in both Red Delicious and Stayman storage apples, it caused only slight damage to freshly harvested Red Delicious apples and none at all to Stayman. To obtain damage on fresh fruit comparable with that produced on storage apples by a 2-hour exposure to 0.33% NH_3 required a concentration in excess of 0.50% NH_3 for 2 hours. At this high concentration the skin of the Golden Delicious apples became brown, not only in areas confined to the lenticels but all over the fruit. Whether this increased susceptibility of apples following a storage period can be attributed to any of the progressive changes that occur, such as decrease in acidity or a change in the pectin fractions associated with softening of the fruit, cannot be stated at this time (2, 5).

The characteristic response of peach following ammonia fumigation was an all-over blackening which faded at the lower rates of fumigation but which progressed to a brownish "bruised" appearance at the higher rates of fumigation (Fig. 2, below). Such varieties as Blake and Red Crest tended to show discrete spots as well as the larger "bruised" patches (Fig. 2, above). Among the three varieties tested (Table 2), there was no apparent difference in susceptibility to ammonia. As a group, however, peaches were more sensitive than apples. Fumigation at 0.04% for 6 hours, which injured peach, left apple unmarked. Fumigation at 0.33% induced severe permanent damage to peach within 1 hour, whereas apples required 2 hours for moderate injury to occur.

Total nitrogen analyses were made of the peelings to determine whether chemical tests could be used to detect accidental fumigation of the fruit in storage (apples, Tables 3 and 4). At the lower fumigations there was no apparent increase in total nitrogen content of any of the apple varieties. At the highest fumigation levels there was an immediate increase in total nitrogen, but it did not persist for more than a few days. There was no apparent difference between the absorptive capacity of stored and freshly picked apples at comparable fumigations. Data for nitrogen content of peach skins are presented in Table 5. Peach absorbed considerably more ammonia than did apple under similar circumstances. In addition, there was significant ammonia absorption even at the lowest fumigation, especially in Blake and Red Crest, but, as with apple, the elevated nitrogen level decreased after several days. Results of the nitrogen determinations would, therefore, be of significance only if the tissue were analyzed soon after fumigation.

In an effort to explain varietal differences in apple, the lenticels were studied microscopically. Lenticels on the fruit are formed beneath stomatal openings left by fallen trichomes or breaks. There are no distinct masses of periderm formed (1). When the lenticels of the five apple varieties were studied, no characteristic difference could be observed in size of the lenticels. Tests with Sudan IV for suberin were positive for all varieties, and tests with phloroglucinol for gum were negative for all varieties. There was a variation in the number of lenticels counted in a given area (200 sq. mm): Red Rome had an average of 37 lenticels per 200 sq. mm; Stayman, 26; Golden Delicious, 18; and Red Delicious, 11. Lenticel number, however, did not appear responsible for the observed differences in varietal response.



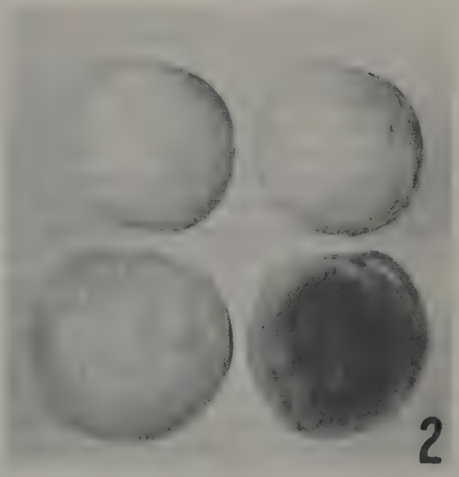


FIGURE 1. Injury to Rome apple caused by exposure to ammonia gas: Above left, uninjured fruit. Right, appearance of fruit immediately after fumigation. Below, appearance of fruit 24 hours after fumigation.

FIGURE 2. Injury to peach fruit caused by exposure to ammonia gas: Above left, uninjured Blake peach. Right, appearance of peach after fumigation showing discrete spots. Below left, uninjured Elberta peach. Right, appearance of peach after fumigation showing all-over blackening or "bruising."

This type of lenticel injury on apple is not to be confused with another symptom sometimes found in storage. Where certain antioxidants have been used in high dosage for scald prevention, an injury pattern develops which may involve the area of the lenticels but which does not in all cases originate at the lenticel. Moreover, this latter-type injury has a black color resembling the immediate, transitory ammonia response, but it persists, whereas the ammonia injury either passes on to a second "corky" stage or fades completely.

There are strong indications that pH or total acidity is involved in the manner in which ammonia affects apple and peach tissue. Although preliminary investigations of titrable acidity of stored apples have failed to explain the varietal differences in susceptiblity to ammonia, further investigation of the buffer system may throw more light on the situation.

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VIRAL COMPOSITION OF FEATHERY MOTTLE IN GEORGIA RED SWEETPOTATO

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Summary

This study on natural transmission demonstrated that var. Georgia Red source of feathery mottle, called mosaic in Georgia, when tested on sweetpotato indicators in Beltsville was a mixture of three virus diseases -- yellow dwarf, internal cork, and leafspot -- confirming earlier findings on the original Beltsville source.

The field observations during 1960 gave little evidence of what had taken place within the naturally inoculated healthy plants from planting time in May until the end of the growing season in October, except for the leafspot virus component which was already much in evidence in July. In the greenhouse, the first new sprout growth displayed: (a) the dwarfing, systemic vein-clearing symptoms of yellow dwarf virus and (b) the chlorotic leafspots of the internal cork virus syndrome.

As an assaying host for feathery mottle, the multisusceptible variety Porto Rico displayed symptoms of the virus complex as follows: (a) leafspot in the field during the growing season in July and a trace of yellow dwarf in mid-September; (b) yellow dwarf on the first new sprout growth in the greenhouse during November-December produced by bedding roots following harvest and curing in October, plus the chlorotic foliage symptoms of the internal cork virus syndrome; and (c) the presence of internal cork lesions in roots after 6 months' warm storage (70° C, 85% RH).

At the conclusion of this study, rows one and two of multisusceptible var. Porto Rico showed 27. 3 and 76. 7% yellow dwarf, 38. 6 and 41. 8% internal cork, and 88. 6 and 73. 8% leafspot. Rows 4 to 6 of La. IX seedling showed 64. 0, 16. 0, and 42. 5% yellow dwarf; 18. 0, 18. 0, and 16. 0% internal cork; 18. 0, 0. 0, and 10. 6% leafspot.

Rows 8 to 10 of La. XXI seedling showed 16. 2, 4. 5, and 20. 4% yellow dwarf; 0. 0, 2. 2, and 2. 2% internal cork; and 2. 3, 6. 8, and 9. 0% leafspot. Rows 12 and 13 of La. I seedling showed 5. 1 and 0. 0% yellow dwarf, 0. 0 and 0. 0% internal cork, and 40. 4 and 35. 4% leafspot.

INTRODUCTION

An earlier report (4) demonstrated that the Beltsville, Maryland source of sweetpotato feathery mottle was a mixture of three virus diseases: yellow dwarf, internal cork, and leafspot. The several sources of yellow dwarf virus studied earlier (Beltsville feathery mottle, California feathery mottle, California yellow dwarf, and New Jersey yellow dwarf) were believed identical because all were graft- and whitefly-transmissible, have the same incubation periods, and produce the same vein-clearing symptoms on indicator plants in the absence of other viruses.

This report gives the results of the 1960-61 study on the natural transmission of Georgia feathery mottle virosis under the growing conditions at Beltsville. The Georgia source of feathery mottle was observed in var. Georgia Red at Tifton in July 1959, when it was arranged to obtain and subject this material to the year-long testing procedure required for determining the viral components of the feathery mottle complex present in Georgia.

MATERIALS AND METHODS

Viral source materials for this experiment were var. Georgia Red roots of the 1959 crop received from H. W. Rankin at Tifton, Georgia.

This experiment was run in a plot consisting of 13 rows of 50 plants each, spaced 1 foot apart, in rows 4 feet wide. The three diseased rows, No. 3, 7, and 11, were planted with var. Georgia Red affected with so-called Georgia mosaic. The remaining 10 rows received virus-free plants of four sweetpotato indicators as follows: Rows 1-2, Porto Rico (Chase, La.); Rows 4-6, seedling La. IX; Rows 8-10, seedling La. XXI; and Rows 12-13, seedling La. I. These sweetpotato varieties and indicators were selected for showing disease symptoms as follows: var. Porto Rico was used because it is susceptible to all three components of the feathery mottle syndrome; seedling La. IX is susceptible to yellow dwarf (YD) and internal cork (IC), but resistant to leafspot (LS); seedling La. XXI is susceptible to yellow dwarf, but resistant to internal cork and leafspot; seedling La. I is susceptible to leafspot, klendusic to yellow dwarf, and resistant to internal cork. The experimental plants were grown in an isolated field to prevent inoculum being brought in from other sources.

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Indian mallow (Abutilon theophrasti) was interplanted in the diseased rows to build up a large population of the abutilon whitefly, Trialeurodes abutilonea (Hald.). In mid-August the weeds were cut and laid on the diseased rows for a few days. The abutilon whiteflies became viruliferous during the larval stage and by September 1 the viruliferous adults were present to disseminate the yellow dwarf virus to adjoining rows of healthy plants.

The whitefly vector of yellow dwarf overwinters in the pupal stage at Beltsville and first became evident on its weed host in late June or early July and requires at least a month or until past midsummer to build up a population adequate for vector action and transmission of yellow dwarf virus. This is why yellow dwarf virus does not ordinarily show current symptoms in Maryland. Hence, the incidence of yellow dwarf cannot be determined until after harvest.

The aphid vectors were active early in the growing season and by mid-July symptoms of the internal cork-leafspot syndrome were evident. Field observations were made at weekly intervals to record the incidence of foliage symptoms.

For greenhouse determination, single roots from each hill were bedded in sand immediately following harvest and curing in October. Five sprouts were planted in 4-inch pots for subsequent observation of symptoms. The identity of the leafspot component in the sprouts from the bedded roots was readily ascertained by means of the manual "squeeze-rub" rapid transmission technique (3), employing the Scarlett O'Hara morning glory indicator. Usually within a month from potting, the "flush of growth" stage of sweetpotato development (2) will reveal the presence of any or all sweetpotato viruses, regardless of their identity.

The remaining roots were held in warm storage (70° F, 85% RH) for 6+ months or until about May 1, 1961; and then sliced for readings on root lesions. Thus, although the aphid vector was active and transmitted the internal cork virus early in the 1960 growing season, it was not possible to determine what had taken place until almost a year later.

EXPERIMENTAL RESULTS

Field Observations: Field observations were of little value in detecting plants naturally inoculated with feathery mottle, except for the leafspot virus component which was much in evidence in July. Seedling La. I indicator, which is susceptible to leafspot, but resistant to internal cork, served a useful purpose in distinguishing the leafspot component more precisely than if the multisusceptible var. Porto Rico alone had been used, as in the past.

The first symptoms to appear on new sweetpotato foliage in the field were chlorotic spots during July, when these symptoms appeared on var. Porto Rico in Rows 1 and 2, and on La. I seedling in Rows 12 and 13 (Table 1). Rows 1 and 2 of var. Porto Rico showed 28 and 26% foliage symptoms in July, compared with 38 and 34% for Rows 12 and 13 of leafspot-susceptible La. I seedling.

Current-season symptoms of yellow dwarf virus rarely appear in the field in this part of the United States. Two plants of sweetpotato seedling La. XXI, however, were showing early yellow dwarf symptoms in mid-September 1960, resulting from inoculation by the abutilon whitefly around September 1 after an incubation period of about 2 weeks.

Greenhouse Observations: The percentage incidence of yellow dwarf virus in the four sweetpotato indicators is recorded by rows in Table 1.

Table 1 and Figure 1 indicate an extremely limited flight range for the adult viruliferous abutilon whitefly. In other words, the percentage incidence of yellow dwarf for var. Porto Rico dropped from 76.7 to 27.3% in the 4-foot width between Rows 2 and 3. Comparable figures for La. IX were drops from 64.0 and 42.5 to 16.0%; for La. XXI, drops from 16.2 and 20.4 to 4.5% and for LA. I, from 5.1 to 0.0%.

Yellow dwarf virus by itself produces only vein-clearing and dwarfing symptoms. In this experiment, however, none of the multisusceptible var. Porto Rico plants were infected with yellow dwarf virus alone. Instead, the vein-clearing and dwarfing symptoms were always accompanied by chlorotic foliage symptoms which characterize the internal cork syndrome.

The La. XXI seedling, although very susceptible to yellow dwarf virus, showed a lower incidence than for var. Porto Rico and La. I seedling. For this, there is no ready explanation. La. I seedling, while susceptible to yellow dwarf through graft inoculation, appears klendusic to whitefly inoculation, under the conditions of this experiment.

Laboratory Observations: The amount of internal cork in the 1960 planting is shown in

Table 1. Results of the 1960 study on the incidence in four sweetpotato indicator plants of the three virus components present in the Georgia source of the feathery mottle virus syndrome.

Row	Nature of plant material	Field plants at end of experiment	Virus incidence ratings			
			YD[a] Gh (%)	IC[b] Lab (%)	LS[c] Field (%)	LS[d] Gh (%)
1	Porto Rico	44	27	39	28	89
2	Porto Rico	43	77	42	26	74
3	Georgia Red	46	100	20	--	--
4	La. IX	50	64	18	0	18
5	La. IX	50	16	18	0	0
6	La. IX	47	43	16	0	11
7	Georgia Red	48	100	--	--	--
8	La. XXI	43	16	0	0	2
9	La. XXI	44	5	2	0	7
10	La. XXI	44	20	2	0	9
11	Georgia Red	36	100	--	--	--
12	La. I	47	5	0	38	40
13	La. I	48	0	0	34	35

[a] Greenhouse sand bed observations in November-December 1960 of yellow-dwarf incidence in sprouts developed on storage roots from the 1960 field harvest.

[b] Incidence of cork lesions when storage roots were sliced after 6 months' warm storage, April 20, 1961.

[c] Chlorotic spot symptom incidence noted in observations of plants in field plots in July 1960.

[d] Leafspot incidence in sprouts produced in greenhouse on storage roots from 1960 field-harvested roots when plants were inoculated by "squeeze-rub" transmission technique -- observations in November-December 1960.

column 4 of Table 1. The incidence of root lesions averaged about 40% for multisusceptible var. Porto Rico, 18% for cork-susceptible La. IX, 2% for cork-resistant La. XXI, and 0.0% for cork-resistant La. I.

DISCUSSION AND CONCLUSIONS

In discussion of this experiment, it should be constantly borne in mind that feathery mottle is a mixture of three virus components of indeterminate proportionality. It should also be noted that whenever plants failed to produce roots, they appear as blanks in Figure 1, which summarizes the health status of the roots of each plant at the end of the experiment.

In addition, the changes that took place in the field at Beltsville could conceivably be quite different from what might have occurred in Georgia where the sweetpotato whitefly vector of yellow dwarf virus occurs (1). Wherever such a test might be conducted, however, the pattern of dissemination would be the sum total of factors conditioning dissemination, *per se*: 1) host reaction to the component viruses, 2) host preference by the insect vectors, 3) the kind and number of vectors present and the timing of their activity, 4) the proportionality of the virus components in the inoculum, 5) the weather conditions that prevailed, and 6) the congeniality of the four sweetpotato indicators to the feathery mottle virus complex, individually and severally.

Of the four indicators used only multisusceptible var. Porto Rico accepted and reacted positively to the three virus components of feathery mottle from multisusceptible var. Georgia Red inoculum source, as shown in Table 1.

The incidence of internal cork in the inoculum source of diseased Georgia Red was 19.5% for Row 3, which is about half as large as for multisusceptible Porto Rico. The La. I seedling proved most valuable for demonstrating the early season spread of the leafspot virus component of feathery mottle. For example, the average incidence rating for leafspot of 36% in the field in July 1960 remained unchanged at the end of the experiment in May 1961, 9 months later.

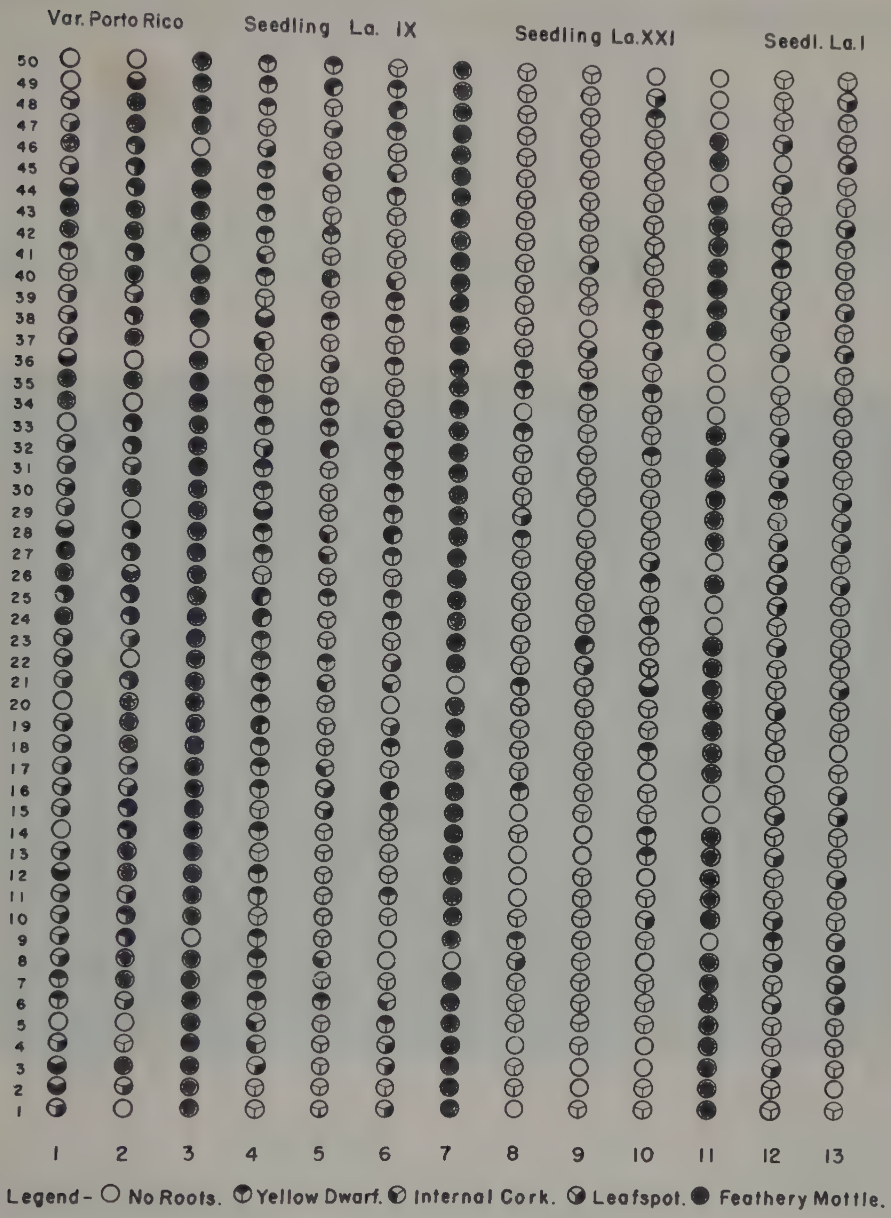

FIGURE 1. Map of 1960 feathery mottle planting showing the disease status of the plants at the end of the experiment.

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AN ECTOPARASITIC ROOT NEMATODE ON LOWBUSH BLUEBERRY (VACCINIUM SPP.)[1]

G. T. Morgan and G. W. Wood[2]

Little information is available on primary nematode parasites of lowbush blueberry roots. A survey of stylet-bearing nematodes in the northeastern United States reports species of 19 genera collected from blueberry areas (1). The only report of pathogenicity of root nematodes has been negative (2), wherein it was shown that *Pratylenchus penetrans* (Cobb) was not a primary parasite of lowbush blueberry.

As part of a study to determine the role of parasitic nematodes in depressed yields of blueberry in the Maritime Provinces, this is to report *Helicotylenchus* sp. (near *H. platyurus* Perry et al., 1959)[3] as an ectoparasite on blueberry roots and rhizomes. The species was found consistently in all areas sampled. (Fig. 1).

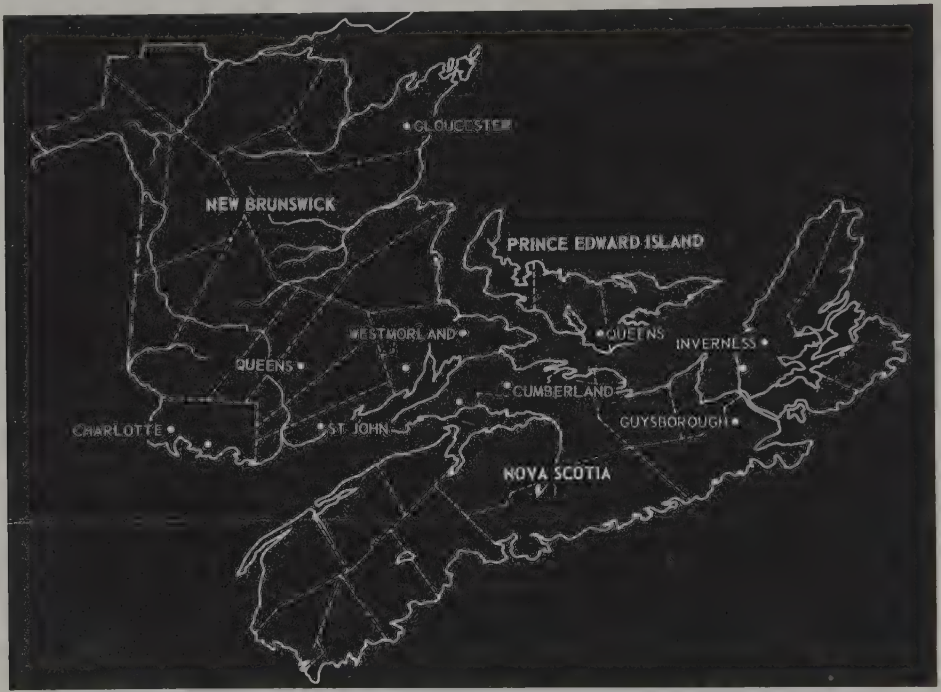

FIGURE 1. Distribution of *Helicotylenchus* sp. in commercial lowbush blueberry areas in the Maritime Provinces.

Helicotylenchus sp. was recovered from all samples both from the rhizosphere and from roots. Individuals were also observed feeding *in situ* on the less woody portions of the roots.

The blueberry seems to be a favored host of the species, although a number of weed species collected with the blueberry samples may be attractants.

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EFFECT OF DATE OF PLANTING ON FUNGI AND OTHER MICROORGANISMS ISOLATED FROM COTTON SEEDLINGS

Hosni A. Mohamed[1]

Summary

Fungi isolated from cotton seedlings were, *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium bataticola*, *Helminthosporium* spp., *Cladosporium* spp., *Aspergillus* spp., and *Alternaria* spp. None of the isolates of *Fusarium* was of the type causing Fusarium wilt of cotton. Percentages of these fungi differed in the 16 dates of planting. Bacteria and nematodes were isolated in certain plantings but not in others.

Some cotton diseases cause great losses in yield and sometimes are the limiting factor in cotton production. Most of these diseases are primarily caused by soil microorganisms.

It is therefore recommended in the U. A. R. that cotton be planted later in the North than in the South. This is primarily to escape certain of these diseases and minimize the hazard caused by them.

Information concerning fungi and other microorganisms that attack cotton seedlings over a period of time is needed. Previous studies in the U. A. R. are few and are limited to diseased seedlings (1, 3).

MATERIALS AND METHODS

The two cotton varieties selected for this study were Bahtim 185 and the line Bahtim 190. The first is highly resistant to Fusarium wilt and the second is very susceptible to the disease.

Seeds of the two cotton varieties were planted in the field at Bahtim on 16 dates of planting at weekly intervals from January 15 to April 28, 1960. Random samples were taken after 53 days from planting, measured, and average length calculated. Infection with Fusarium wilt was considered and notes were recorded. Seedlings were taken to the laboratory, roots were cut 1 cm long, surface disinfected with 0. 1% mercuric chloride and then plated on potato-dextrose agar. Petri dishes were incubated at 25° C and notes taken after 10 to 15 days. Fungi were identified, counted and then the percentage of each fungus to the total number of fungi was calculated. Presence or absence of both bacterial colonies or nematodes was also noted.

Minimum, maximum and average air temperatures were calculated for each date of planting from planting till samples were taken (Table 1).

RESULTS AND DISCUSSION

Effect on Length of Seedlings: Length of seedlings was measured 53 days from planting when samples were taken for laboratory studies. Table 1 shows the average length of seedlings. Length of seedlings increased with the progress of the season or in other words with the increase in temperature until the March 25th planting; after this, the length decreased.

Effect on Fungi Isolated from Cotton Seedlings: Fungi were identified 10 to 15 days from plating on the artificial media. Number of colonies of each fungus was related to the total number of fungi for each date of planting (Tables 2 and 3).

Tables 2 and 3 show that fungi isolated were, *Fusarium* spp., *Rhizoctonia solani* Kuehn, *Sclerotium bataticola* Taub., *Alternaria* spp., *Helminthosporium* spp., *Aspergillus* spp., and *Cladosporium* spp. All these fungi can live saprophytically in the soil, but some of them, such as *R. solani* and certain species of *Fusarium*, can parasitize cotton plants. Percentage of fungi isolated differed in the different dates of planting.

Isolates of *Fusarium* spp. were isolated in all plantings except in that of April 22. Percentage of isolates fluctuated from one planting to another. The trend differed in the two cotton varieties.

In general, isolates of *Fusarium* comprise the larger percentage among the fungi isolated. This genus is especially important because it includes *Fusarium oxysporum* f. *vasinfectum* Snyder & Hansen, the cause of Fusarium wilt, the most important disease of cotton in Egypt. To detect the presence of this fungus in the soil, the line Bahtim 190 was included in the exper-

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Table 1. Average, maximum and minimum air temperatures in the period from planting until samples were collected for 16 dates of planting at Bahtim 1960, and average length of seedlings after 53 days.

	Dates of sowing															
	Jan. 15	Jan. 22	Jan. 29	Feb. 5	Feb. 12	Feb. 19	Feb. 26	Mar. 4	Mar. 11	Mar. 18	Mar. 25	Apr. 1	Apr. 8	Apr. 15	Apr. 22	Apr. 28
Air temperature in ° C:																
Average	14.1	14.2	15.2	16.0	16.9	17.4	18.2	19.4	20.1	21.6	22.1	22.8	24.1	25.0	25.8	26.0
Maximum	31.4	31.4	32.2	35.2	38.6	38.6	38.6	38.6	39.1	40.7	40.7	40.7	40.7	40.7	41.2	41.2
Minimum	1.4	1.4	2.3	2.3	5.1	5.1	5.1	5.1	6.6	8.0	8.0	8.3	8.3	9.6	9.6	9.6
Av. length of seedlings in cm:																
Bahtim 185	12.5	13.3	16.1	23.4	24.1	22.8	25.9	----	39.4	43.5	44.6	30.6	----	----	----	----
Line 190	13.1	17.0	18.8	22.9	22.4	23.8	26.8	----	35.5	45.5	44.8	34.5	----	----	----	----

Table 2. Fungi, bacteria, and nematodes isolated from seedlings of the cotton variety Bahtim 185 planted at 15 dates of planting.

Fungi, bacteria and nematodes	Percentage of fungi isolated when sown														
	Jan. 15	Jan. 22	Jan. 29	Feb. 5	Feb. 12	Feb. 19	Feb. 26	Mar. 4	Mar. 11	Mar. 18	Mar. 25	Apr. 8	Apr. 15	Apr. 22	Apr. 28
Fusarium spp.	48.9	54.2	23.5	17.5	34.3	34.9	40.5	36.4	75.0	27.3	6.3	17.2	14.9	----	18.2
Rhizoctonia solani	6.4	1.4	----	----	1.4	2.3	----	----	----	----	8.3	13.8	4.3	----	----
Sclerotium bataticola	----	4.2	----	1.8	11.4	27.9	21.4	33.3	----	----	----	1.7	----	----	----
Helminthosporium spp.	6.4	4.2	5.9	8.8	14.3	11.6	14.3	----	----	12.1	2.1	12.1	----	----	----
Alternaria spp.	27.7	25.0	39.7	31.6	7.1	23.3	11.9	12.1	21.9	60.6	2.1	10.3	17.0	----	----
Cladosporium spp.	----	2.8	25.0	24.6	21.4	----	----	----	3.1	----	2.1	6.9	----	----	----
Aspergillus niger	6.4	4.2	2.9	10.5	10.0	----	9.5	18.2	----	----	31.3	29.3	51.0	83.3	72.7
Aspergillus spp.	----	----	----	5.3	----	----	2.4	----	----	----	45.8	8.6	12.8	16.7	9.1
Unidentified fungi	4.3	4.2	2.9	----	----	----	----	----	----	----	2.1	----	-----	----	----
No. of bacterial colonies	----	----	----	----	----	----	3	1	21	----	----	5	----	----	----
Presence of nematodes[a]	P	P	P	P	P	----	----	S	----	----	----	----	----	----	----

[a] P = present, S = scarce.

Table 3. Fungi, bacteria, and nematodes isolated from seedlings of the cotton line Bahtim 190 planted at 16 dates of planting.

Fungi, bacteria and nematodes	Percentage of fungi isolated when sown															
	Jan. 15	Jan. 22	Jan. 29	Feb. 5	Feb. 12	Feb. 19	Feb. 26	Mar. 4	Mar. 11	Mar. 18	Mar. 25	Apr. 1	Apr. 8	Apr. 15	Apr. 22	Apr. 28
Fusarium spp.	28.6	52.6	32.4	35.4	44.8	29.2	21.9	31.7	47.7	7.3	18.4	54.0	18.5	40.4	----	10.4
Rhizoctonia solani	----	----	----	----	----	8.3	6.3	22.0	----	29.3	44.7	----	5.6	----	----	----
Sclerotium bataticola	1.8	----	----	----	----	12.5	----	----	18.2	2.4	----	----	----	1.9	6.1	----
Helminthosporium spp.	10.7	18.4	18.9	12.5	16.4	----	12.5	7.3	20.5	9.8	----	2.5	1.9	1.9	----	----
Alternaria spp.	51.8	23.7	40.5	6.3	23.9	20.8	31.3	17.1	----	31.7	5.3	10.0	13.0	----	----	43.8
Cladosporium spp.	----	----	5.4	16.7	7.5	4.2	----	----	9.1	----	----	7.5	9.3	3.8	----	----
Aspergillus niger	----	----	2.7	29.2	----	25.0	12.5	22.0	----	12.2	15.8	30.0	50.0	57.9	45.5	33.3
Aspergillus spp.	1.8	2.6	----	----	6.0	----	3.1	----	4.5	7.3	15.8	5.0	1.9	----	48.5	12.5
Unidentified fungi	5.4	2.6	----	----	1.5	----	12.5	----	----	----	----	----	----	----	----	----
No. of bacterial colonies	----	----	----	----	----	----	2	11	14	----	----	10	----	----	----	----
Presence of nematodes[a]	P	P	P	P	P	----	P	----	----	----	----	----	----	----	----	----

[a] P = present.

iment. In the meantime almost every culture of *Fusarium* isolated was increased separately and pathogenicity tests were made. None of the isolates tested seemed to be of the type causing Fusarium wilt of cotton. This was confirmed in field tests when none of the seedling or adult plants of either Bahtim 185 or Bahtim 190 showed symptoms of Fusarium wilt.

R. solani was isolated in small percentages in some plantings but not in others. It is not surprising that it was isolated from late plantings when temperature was high (Tables 2 and 3), as temperature requirement differed according to the different isolates (2). *R. solani* is considered the most important fungus among those causing damping-off of cotton.

Since *S. bataticola* was isolated often, especially in the middle of the season, it would appear to be an important fungus on cotton; however, its importance has not yet been clarified.

Other fungi isolated were mostly saprophytes that have not been proved to be parasitic on cotton. Most of these fungi were isolated over the entire period with fluctuating percentages.

Effect on Other Microorganisms: In the course of identification of fungi, presence and absence of bacterial colonies and nematodes was recorded. No attempt was made to identify either the bacteria or the nematodes. Tables 2 and 3 show that bacterial colonies were isolated from cotton planted in the middle of the period, while nematodes were found in plants from early plantings. Thus, temperature in the middle of the period favored an increase of bacteria in the soil and temperature in early plantings favored an increase of nematodes.

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INVESTIGATION OF THE ABILITY OF GEOTRICHUM CANDIDUM AND TRICHODERMA VIRIDE TO SPREAD BY CONTACT INFECTION IN NONVENTED VERSUS VENTED CARTONS OF LEMONS

Paul R. Harding and Donald C. Savage[1]

Abstract

In the case of Trichoderma viride Pers. ex S. F. Gray, a fungus which is partially sensitive to biphenyl, the nonvented carton was not more conducive than the vented carton to the spread of decay by contact infection in lemons when biphenyl was used. However, when biphenyl treatment was not used, the nonvented carton was more conducive to the spread of T. viride through contact infection than was the vented carton. In the case of Geotrichum candidum Link ex Pers., a fungus which is resistant to biphenyl, the nonvented carton was more conducive than the vented carton to the spread of decay in lemons by contact infection with or without biphenyl treatment.

INTRODUCTION

Two types of cartons, namely, vented and nonvented, are in use by the California citrus industry. The wall of each type is comprised of three layers of fiberboard paper material: a corrugated middle layer on which are glued inner and outer flat layers. Both cartons have inside dimensions of 16 3/8 x 10 11/16 x 10 1/4 inches, and are of the "telescopic" type in which the top part slides completely over the vertical sides of the bottom part. The vented carton differs from the nonvented carton only in having 18 round holes, each of which is 1.0 inch in diameter, in the walls of the closed carton. These holes are arranged so that there are six in the top, six in the bottom, and three on each side.

Commercial biphenyl treatment consists of two sheets of No. 48 Crown-Kraft paper impregnated with a mixture containing biphenyl. One sheet is placed in the bottom and the other in the top of the carton. Each sheet has six holes to match the holes in a vented carton. Each sheet is 10 9/16 x 16 1/4 inches and contains 2.35 grams of biphenyl in a mixture of biphenyl 50%, paraffin 25%, mineral oil 24%, and lemon oil 1%.

In August and September of 1957, sour rot, caused by Geotrichum candidum, became unusually troublesome in shipments of lemons received in Hamburg, Germany. At that time, only the nonvented type of carton was in use and the possibility of reducing sour rot by changing to a vented carton for export was considered. The vented carton was tried in some shipments, and by 1958 use of the vented carton became mandatory, because the use of this carton appeared to stop the spread of the fungus by contact infection.

The work of Rygg, et al. (4) indicates that even in long-term shipments biphenyl is more effective in nonvented cartons when biphenyl-sensitive strains of Penicillium digitatum Sacc. and Penicillium italicum Wehmer are concerned. The possibility may exist, however, that a biphenyl-resistant mold such as Geotrichum candidum could spread more rapidly through fruit in nonvented than in vented cartons. Owing to its higher relative humidity (2), the nonvented carton may favor contact infection when molds are present that are resistant to biphenyl treatment. Trichoderma viride is partially resistant to biphenyl and sometimes causes severe losses in export shipments. The object of this investigation was to see if the nonvented carton is more conducive than the vented carton to contact infection in lemons by G. candidum and T. viride.

Consideration was given to the matter of selecting reasonable storage conditions for this experiment. Seven weeks may elapse between the packing of lemons in California and the final distribution to consumer markets in Europe. Following packing, the cartons of lemons are transported directly to the harbor where storage and loading may take 1 week. During that time the fruit is without refrigeration. The trip overseas from the ports of Los Angeles and Long Beach to the ports of Le Havre, Antwerp, and Hamburg normally requires 28 to 30 days. In a suitable ship's hold, the fruit is brought in 3 or 4 days from a temperature of about 70° F to 50°-55° (4); 52° is the temperature recommended for lemons. Unfortunately, however, such desirable refrigeration may not always be provided. After arrival in Europe, 2 weeks may

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Table 1. Effect of nonvented versus vented cartons on the ability of *Geotrichum candidum* to spread by contact infection in lemons stored 6 weeks at 70° F. "A" signifies nonvented, "B" vented.

Carton no.	Treatment	No. fruits	No. decays by contact	% decays by contact	Miscellaneous decays[a]
1A1	biphenyl	153	24	15.69	0
1A2	do.	do.	29	18.95	1 blue
1A3	do.	do.	29	18.95	1 blue, 2 green
1A4	do.	do.	34	22.22	0
1A5	do.	do.	36	23.53	0
				Av. 19.868	
1B1	biphenyl	153	16	10.46	1 blue
1B2	do.	do.	17	11.11	1 blue
1B3	do.	do.	22	14.38	2 blue
1B4	do.	do.	21	13.73	0
1B5	do.	do.	15	9.80	0
				Av. 11.896	
2A1	none	153	36	23.53	3 blue
2A2	do.	do.	63	41.18	1 blue
2A3	do.	do.	40	26.14	3 blue
2A4	do.	do.	40	26.14	2 blue, 2 green
2A5	do.	do.	70	45.75	0
				Av. 32.548	
2B1	none	153	19	12.42	2 blue
2B2	do.	do.	8	5.23	2 blue
2B3	do.	do.	10	6.54	2 blue
2B4	do.	do.	16	10.46	2 blue
2B5	do.	do.	26	16.99	0
				Av. 10.328	

[a] Decays other than by *G. candidum*. Blue mold is *Penicillium italicum*; green mold is *Penicillium digitatum*.

elapse before the cartons of lemons are distributed to consumer markets. During that time the fruit is without refrigeration. In a transportation test using noninoculated fruit, obtaining statistically significant results to answer the question of desirability of vented cartons over nonvented cartons is impossible unless lemons of low keeping quality are used (4). When such a test is prepared, the keeping quality of lemons can only be estimated, and prediction is impossible that the lemons will decay in sufficient numbers to give statistically significant results. Moreover, in a transportation test the fruit encounters wide variations in temperature and relative humidity, making comparison of one test with another or even the data within a given test difficult. In the present tests, which are of a static nature, we have contrived to obtain statistically significant results by using fixed conditions of storage, and by exposing lemons by contact to a specific number of inoculated fruits. Our desire was to study contact infection by *G. candidum* and *T. viride* in vented and nonvented cartons of lemons under refrigerated and nonrefrigerated conditions. We chose 50° F and 85% relative humidity for refrigerated conditions, and 70° and 50% relative humidity for nonrefrigerated conditions.

MATERIALS, METHODS, AND RESULTS

Packing-house processed lemons of sound appearance and of proper color and texture for commerce were randomized in the process of filling 40 cartons, 20 of the nonvented type and 20 of the vented type. The lemons were of such size as to give 165 fruits per carton. A hypodermic syringe was used to inoculate 12 fruits in each carton. The inoculated fruits were distributed randomly amid the sound fruits. Inoculation was performed, after surface sterilization with 70% alcohol, by inserting the hypodermic needle at an angle between the albedo and juice pulp and injecting 1 ml of concentrated (about 1 million spores per ml) spore suspension of *G.*

Table 2. Effect of nonvented versus vented cartons on the ability of *Trichoderma viride* to spread by contact infection in lemons stored 6 weeks at 70° F. "A" signifies nonvented, "B" vented.

Carton no.	Treatment	No. fruits	No. decays by contact	% decays by contact	Miscellaneous decays[a]
1A1	biphenyl	153	7	4.58	1 blue
1A2	do.	do.	9	5.88	0
1A3	do.	do.	7	4.58	0
1A4	do.	do.	1	0.65	0
1A5	do.	do.	31	20.26	1 blue, 1 green
			Av.	7.190	
1B1	biphenyl	153	4	2.61	1 blue, 1 green
1B2	do.	do.	0	0.00	0
1B3	do.	do.	2	1.31	0
1B4	do.	do.	10	6.54	2 blue, 1 green
1B5	do.	do.	17	11.11	0
			Av.	4.314	
2A1	none	153	82	53.59	0
2A2	do.	do.	85	55.56	0
2A3	do.	do.	62	40.52	0
2A4	do.	do.	30	19.61	0
2A5	do.	do.	40	26.14	0
			Av.	39.084	
2B1	none	153	11	7.19	0
2B2	do.	do.	20	13.07	3 green
2B3	do.	do.	13	8.50	0
2B4	do.	do.	7	4.58	3 blue
2B5	do.	do.	14	9.15	0
			Av.	8.498	

[a] Decays other than by *T. viride*. Blue mold is *Penicillium italicum*; green mold is *Penicillium digitatum*.

candidum. The following arrangement of cartons was placed in the storage room running at 50° F: five nonvented cartons with biphenyl treatment; five vented cartons with biphenyl treatment; five nonvented cartons without treatment; and five vented cartons without treatment. An identical arrangement of cartons was placed in the storage room running at 70°. The entire foregoing procedure was repeated except that a spore suspension of *T. viride* was used for inoculum. After 6 weeks the cartons of lemons were removed from the storage rooms and examined for decay resulting from contact infection. No contact infections in the case of either mold resulted in the cartons stored at 50°. Data concerning the effect of nonvented versus vented cartons on contact infection in lemons stored at 70° are shown in Table 1 for *Geotrichum candidum* and in Table 2 for *Trichoderma viride*.

STATISTICAL ANALYSIS OF DATA

The Lord (3) range test was used to detect significant differences between averages for answering several important questions. In our work we have considered as valid only mean differences which are significant at no critical value less than that of the 99% confidence level. In Lord's Table 10, the critical value is 0.896 for five replications at 1% error probability or the 99% confidence level.

1. Was the average percentage of decay significantly greater in nonvented than in vented cartons with biphenyl treatment?

Mold	Average range	Difference in means	D. M./A. R.	Significant at 99% Confidence level (0.896)
G. candidum	6.210	7.972	1.284	Yes
T. viride	15.360	2.876	0.187	No

2. Was the average percentage of decay significantly greater in nonvented than in vented cartons with no treatment?

Mold	Average range	Difference in means	D. M./A. R.	Significant at 99% Confidence level (0.896)
G. candidum	16.990	22.220	1.308	Yes
T. viride	22.220	30.586	1.377	Yes

3. Did biphenyl treatment give a significantly lower average percentage of decay than no treatment in nonvented cartons?

Mold	Average range	Difference in means	D. M./A. R.	Significant at 99% Confidence level (0.896)
G. candidum	15.030	12.680	0.844	No
T. viride	27.780	31.894	1.148	Yes

4. Did biphenyl treatment give a significantly lower average percentage of decay than no treatment in vented cartons?

Mold	Average range	Difference in means	D. M./A. R.	Significant at 99% Confidence level (0.896)
G. candidum	8.170	1.568	0.192	No
T. viride	9.800	4.184	0.427	No

DISCUSSION AND CONCLUSIONS

Without biphenyl treatment, the nonvented carton was more conducive than the vented carton to contact infection in lemons by either Geotrichum candidum or Trichoderma viride. With biphenyl treatment, the nonvented carton was more conducive than the vented carton to contact infection in lemons by G. candidum, but not by T. viride. That there was no significant difference between the two types of cartons in the amount of contact infection by T. viride was likely due to the fungus being partially sensitive to biphenyl, together with the lesser loss of biphenyl vapor by the nonvented carton being neutralized by the bad effects of nonventing. On the other hand, G. candidum is completely resistant to biphenyl, so the bad effects of nonventing were not counterbalanced.

Actually, if biphenyl is used, the nonvented carton is less conducive than the vented carton to decay by biphenyl-sensitive molds; for example, normal strains of Penicillium italicum and Penicillium digitatum (1, 4). However, for molds that are partially sensitive to biphenyl, such as T. viride, one type of carton is no more conducive to decay than the other, if biphenyl is used. For molds that are resistant to biphenyl, such as G. candidum, the vented carton is less conducive than the nonvented carton to decay, whether biphenyl is used or not. In general, if no treatment is used the vented carton is less conducive to decay than is the nonvented carton.

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APPLE SCALD AND ITS CONTROL BY THE USE OF DIPHENYLAMINE OR SANTOQUIN[1]

Robert H. Daines[2]

Summary

DPA provides excellent scald and Stayman spot control when used as a dip or in wraps or shredded paper. Oiled papers offer some protection against scald, but not enough to be of use in a commercial program for scald control.

A DPA dip may injure Stayman or Rome apples, but DPA in paper has proven to be safe and effective against apple scald.

Santoquin (Stop Scald) at 2 or 3 pints per 100 gallons, applied as a dip or as a spray on apples as they pass on a roller conveyor, provided excellent control of apple scald, but when this material was applied as a tree spray or in paper wraps control of this physiogenic disease was not satisfactory.

The effectiveness of Santoquin is greatly reduced if the apples are cold (32° F) at the time of treatment. However, the temperature of the dipping solution (40° to 70° F), when used in an instantaneous dip, had no measurable effect on the efficacy of the treatment as measured by scald development.

Injury or fruit-staining from the use of Santoquin was not observed in the experiments reported here.

The anti-scald chemicals are more effective in preventing scald on Stayman and Rome apples than on Red Delicious or Paragon fruits.

Apple scald, a physiogenic disease that occurs after apples have been held in cold storage for several weeks, renders more apples from refrigerated storages unfit for the fresh market than does any other malady. Since control of scald continues to have great economic significance, the improvement of control measures becomes imperative.

This disturbance consists of an initial browning and subsequent death of surface cell layers of the apple. The development of the brown color is greatly accelerated when the fruit is removed from cold storage to a warmer temperature. After a few days at room temperature, fruit that appeared to be healthy on removal from refrigerated storage may become severely scalded. Although affected fruits are still usable, their appearance is rendered so unattractive by the disease that they are not usually acceptable for the fresh market.

In New Jersey, our most susceptible varieties are Paragon, Stayman, Turley and Rome apples; Red Delicious is intermediate in susceptibility and Golden Delicious usually shows little or no injury from this disorder.

An additional disorder, which we call "Stayman spot," has been observed during the last few years. This spot, which occurs where Stayman apples touch each other or the container, develops during late storage and after these apples are removed from storage. The affected area is generally brown, firm in texture, and may be slightly sunken. The browning usually extends more deeply into the apples than does surface scald, affecting not only the apple skin but also a few layers of cortical parenchyma. These spots are often invaded by fungi which may further extend the injury. Repeated isolations and inoculations have failed to disclose the cause of the malady.

REVIEW OF LITERATURE

The work of the early investigators contributed much to our present knowledge of apple scald. Although the exact cause of scald is unknown, Brooks, Cooley and Fisher (1, 2) and others have established its physiogenic origin. They disclosed the effectiveness of oil for control of the disease and advocated good ventilation in the storage house; in addition, they found the first 6 to 8 weeks of the storage period to be the most active period in scald production. As early as 1903 Powell and Fulton (4) suggested delayed harvest as a means of reducing scald. Despite the seriousness of the scald problem, very little information that led to improved control measures over those elucidated by the early workers referred to was made until Smock tested diphenylamine (DPA) and Santoquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) or

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Stop Scald[3] for the control of this disease. Since the work of Fidler (3) and others suggested that volatiles alone were not responsible for scald, Smock (5), considering that oxidative enzymes might be involved, tested several antioxidants by spraying them on trees before harvest, by dipping the fruit into solutions of the materials, and by impregnating a particular one, DPA, into paper used to wrap the fruit. DPA was reported as the most effective material tested. In these experiments DPA as a spray at 1000 ppm was of little or no value, but at 2000 ppm fairly good scald control on Greening was obtained. When DPA was used at 500 to 2000 ppm in dipping solution, good results were obtained. Smock (5) also reported that several materials used as dips at concentrations varying from 250 to 1000 ppm gave little or no scald control. Some of the effective materials were 2,4-dichloranisole, alpha tocopherol, butylated hydroxyanisole, triphenylamine, hydroxylamine, and diphenyl. Although DPA-treated paper gave excellent scald control, injury and off-flavored fruit were produced. Of the other materials tested, Santoquin gave the most encouraging results.

In a more recent publication (6) Smock concludes that DPA is more effective than Stop Scald when used in equivalent amounts. The comparison of most value, however, considers performance and cost of optimum rather than equivalent concentrations.

EXPERIMENTAL METHODS

In our investigation which has been continuous since 1956, the author has tested diphenylamine (DPA), Santoquin (Stop Scald) and other antioxidants impregnated into wraps and shredded papers. In addition, some of these same materials were used as tree sprays, apple dips, or were applied as a spray to apples as they passed along a roller conveyor over which was placed three Andy-Mo brushes in series. The apples were sprayed with five lines of nozzles and the wet brushes assured a thorough coverage.

In the early experiments (1958, 1959) powdered Santoquin was solubilized in ethyl alcohol and this solution mixed with water to which had previously been added 0.01% Igepal to insure good wetting of the treated apples. DPA dips and sprays were prepared in the same manner in all tests reported here.

DPA impregnated paper wrappers were secured from the United Paper Company. Some were used as wraps and some were shredded. All DPA impregnated paper used in these tests contained the indicated amount of DPA in a 10 by 10-inch wrapper.

The apples used in these experiments were poorly colored and were gathered at the time of the first picking; such apples were used to insure abundant scald. These apples were stored after treatment in regular and controlled atmosphere storage at temperatures of 32° to 35° F.

Scald and Stayman spot counts were made on removal of the fruit from storage in the late spring. These apples were then held at room temperature for 5 or 7 days, when they were again examined for scald and Stayman spot.

RESULTS

The results of the dip treatments used during the 1956-57 storage period were not encouraging, as none of the treatments provided satisfactory scald control. Perhaps the problem was one of concentration and of thorough wetting of the difficult-to-wet Paragon apples. In the 1957-58 experiments, however, DPA provided excellent control of scald and Stayman spot when used in paper or as dips. In this experiment DPA in paper or as dips provided more highly significant control of scald (Table 1) than did shredded oil paper, which was, in turn, significantly better than no treatment. In the second experiment conducted during the 1957-58 storage season, shredded paper impregnated with DPA again gave more highly significant control of scald than did the shredded oil paper, which was, in turn, a significant improvement over the absence of paper. These results applied equally well for scald and Stayman spot.

In 1958-59 experiments, Stayman apples were again treated not only with DPA impregnated shredded paper but with dips of DPA (1000 and 2000 ppm) and Santoquin at the same concentrations (Table 1). The data indicate that the materials fall into seven groups with reference to the main effect of the chemicals. The best control of scald was provided by DPA 2000 ppm as a dip and a Santoquin dip of the same concentration. The next most effective treatment was DPA-2 mg in 100 square inches of shredded paper, followed by a dip of 1000 ppm DPA in shredded paper. A Santoquin dip at 1000 ppm was not so effective as the DPA paper treatments.

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[3] The commercial preparation Stop Scald is an emulsion containing 70% Santoquin.

Table 1. Effect of DPA applied as dip or in paper and Santoquin applied as dip or as a tree spray on storage scald of Stayman apples.

Treatment	Apples showing scald 7 days out of storage											
	1957-58			1957-58			1958-59			1960-61		
	percent	angle[a]	5%*	percent	angle	5%	percent	angle	5%	percent	angle	5%
1. DPA dip, 2000 ppm	3.3	10.5	1				4.8	12.7	1	1.3	6.6	1
2. DPA dip, 1000 ppm	10.0	18.4	2,3,4				11.3	19.6	2,3	4.0	11.5	1,2
3. Santoquin (Stop Scald) dip, 3000 ppm										0.9	5.6	1
4. Santoquin dip, 2000 ppm							7.0	15.3	1,2	2.0	8.1	1,2
5. Santoquin dip, 1000 ppm							25.3	30.2	4	4.4	12.1	2
6. Paper wraps carrying DPA, 2 mg.[b]	3.2	10.3	1	5.2	13.2	1						
7. Shredded paper carrying DPA, 2 mg.	4.8	12.7	1,2				10.4	18.8	1,2,3			
8. Oiled paper wraps carrying DPA, 1.5 mg.	8.0	16.4-	2,3									
9. Paper wraps carrying DPA, 1.5 mg.	5.1	13.1	1,2									
10. Shredded oiled paper carrying DPA, 1.5 mg.	9.8	18.2	3,4	14.9	22.2	1	18.6	25.6	3,4			
11. Shredded paper carrying DPA, 1.5 mg.	14.5	22.4	4	10.1	18.5	1	16.6	24.0-	3,4			
12. Stop Scald tree spray day before harvest, 2 pt.										8.7	16.1	2
13. Shredded oiled paper	43.3	41.2	5	79.0	62.7	2	66.2	54.5	5			
14. Check	72.2	58.5	6	93.4	75.1	2	74.8	59.9	6	29.7	33.1	3

*Treatments followed by the same number do not differ at the 5% level of significance according to Duncan's multiple range test.

[a] Angle = arc sin $\sqrt{\text{percent}}$.

[b] Amount of DPA in 100 square inches of paper.

Table 2. Effect of DPA applied as dip or in paper, and Santoquin applied as dip and spray to Stayman apples of various temperatures on the occurrence of Stayman spot.

Treatment	Apples showing Stayman spot 7 days out of storage								
	1957-58			1958-59			1961-62		
	percent	angle[a]	5%*	percent	angle	5%	percent	angle	5%
1. DPA dip, 2000 ppm				3.3	10.4	1	0.2	2.3	1
2. DPA dip, 1000 ppm				4.1	11.7	1	1.3	6.6	1
3. Santoquin dip, 3000 ppm (3 pt.)							0.0	0.0	1
4. Santoquin dip, 2000 ppm				3.8	11.3	1	2.5	9.1	2
5. Santoquin dip, 1000 ppm				6.9	15.2	2,3			
6. Shredded paper carrying DPA, 2.0 mg.[b]	4.8	12.6	1	4.6	12.4	1,2			
7. Shredded paper carrying DPA, 1.5 mg.	8.5	16.9	1	5.6	13.7	1			
8. Shredded oiled paper carrying DPA, 1.5 mg.	11.4	19.7	1	4.1	11.7	1			
9. Oiled paper wraps	59.0	50.2	2	10.2	18.6	3			
10. Check (untreated)	69.2	56.3	2	11.6	19.9	4	18.1	25.2	3
11. Santoquin 2000 ppm sprayed on apples on roller conveyor with brushes above[c]							6.4	14.6	2
12. Santoquin dip, 2000 ppm[c]							7.4	15.8	2
13. Check (untreated)[c]							15.0	22.8	3

*Treatments followed by the same number do not differ at the 5% level of significance according to Duncan's multiple range test.

[a] Angle = arc sin $\sqrt{\text{percent}}$.

[b] Amount of DPA in 100 square inches of paper.

[c] Apples 32° F at time of application, remaining treatments were at orchard temperatures when dips were made.

Table 4. Stop Scald and DPA applied as dip and spray to apples of various temperatures for scald control 1961-62.

Treatment (per 100 gallons)	Scald count 7 days out of storage											
	Stayman			Red Delicious			Paragon			Red Rome		
	percent	angle[b]	5%*	percent	angle	5%	percent	angle	5%	percent	angle	5%
1. Stop Scald, 3 pt. per 100 gal.	4.9	12.8	1	22.8	28.5	1	38.8	38.5	1	3.4	10.6	1
2. DPA, 2000 ppm	5.9	14.1	1	21.5	27.6	1	46.0	42.7	1,2	4.7	12.5	1,2
3. Stop Scald, 2 pt. solution, 55° F	6.6	14.9	1	45.3	42.3	2						
4. Stop Scald, 2 pt. solution, 40° F	7.4	15.8	1	47.1	43.3	2						
5. Stop Scald, 2 pt.	8.1	16.5	1	48.4	44.1	2	47.6	43.6	1,2	9.2	17.7	1,2
6. Stop Scald, 2 pt. solution, 70° F	11.3	19.6	1,2	44.6	41.9	2	56.1	48.5	2			
7. DPA, 1000 ppm	18.4	25.4	2	48.8	44.3	2	51.8	46.0	1,2	1.3	19.6	2
8. [a]Stop Scald, 2 pt. applied as spray to apples on roller conveyor with series of brushes above apples	65.5	54.0	3									
9. [a]Stop Scald, 2 pt.	86.2	68.2	4									
10. Check (untreated)	87.0	68.8	4	73.2	58.8	3	85.6	67.7	3	91.1	72.6	3
11. [a]Check (untreated)	97.6	81.0	5									

*Treatments followed by the same number do not differ at the 5% level of significance according to Duncan's multiple range test.

[a] Apples 32° F at time of application, remaining treatments were at orchard temperatures when dips were made.

[b] Angle = arc sin $\sqrt{\text{percent}}$.

Although oiled paper provided some control against scald, the protection was inadequate for practical use. The resulting scald was less severe with the DPA and Santoquin treatments than with the other treatments. The percentage of slight scald in each of these instances was considerably greater than was the severe scald, while the reverse was true for the check and oiled paper treatments. The DPA and Santoquin treatments, therefore, not only provided a highly significant reduction in total scald but the apples affected were less severely diseased than were those in the less effective treatments. The excellence of the DPA performance in this experiment was marred, however, by injury to a few apples in the 2000 ppm treatment. In this experiment Santoquin also showed real promise in scald and Stayman spot control (Tables 1 and 2), especially at the higher concentration. The data also indicate that the apples scalded more in regular storage than in the controlled-atmosphere room. This same trend did not always occur; in 1957-58 the reverse was true.

In 1959-60 an experiment was conducted at the New Jersey Agricultural Experiment Station in which DPA and Santoquin dips were compared with each other and with an untreated check for control of scald development on Stayman apples. In this trial 6 bushels of first-harvest Stayman were used in each treatment, and again DPA and Santoquin exhibited real value in scald prevention but DPA injured about 2% of the apples in the 2000 ppm treatment.

In the 1959-60 treatments 45 paper-wrap treatments, each using 4 bushels of green-colored Paragon apples gathered at the first picking, were used (Table 3). These data indicate that the DPA-containing wrappers effected a highly significant reduction in the amount of scald that developed on this scald-susceptible fruit, while the oiled paper wrappers and those treated with Santoquin had only a slight effect on this malady. In addition, butylated hydroxyanisole (2-and 3-isomers), butylated hydroxytoluene, alpha tocopherol, delta tocopherol, and lauric acid amid of para amine phenol provided evidence of assistance on the 5% level. All other materials tested were of no value.

Table 3. The effect of paper wraps impregnated with various chemicals on the occurrence of scald on Paragon apples 1959-60[a].

	Apples showing scald 7 days out of storage			
Treatment	percent	angle[b]	1%**	5%*
1. DPA 4.0 mg. per wrapper	22.3	28.2	A	1
2. DPA 3.0 mg.	59.7	50.6	B	2
3. DPA 2.0 mg.	64.0	53.1	B	2, 3
4. DPA 1.5 mg.	78.5	62.4	B, C	3, 4
5. Santoquin 4 mg.	91.1	72.6	C, D	4, 5
6. Santoquin 3 mg.	92.1	73.7	C, D	5
7. Santoquin 2 mg.	94.5	76.4	D, E	5
8. Oiled paper wraps	94.9	76.9	D, E	5
9. Santoquin 1.5 mg.	95.9	78.3	D, E	5, 6
10. Check (untreated paper wraps)	99.9	88.4	E	6
11. Check (no wraps)	99.9	88.7	E	6

*Treatments followed by the same number do not differ at the 5% level of significance according to Duncan's multiple range test.

**Treatments followed by the same letter do not differ at the 1% level of significance according to Duncan's multiple range test.

[a] Poorly colored apples from first picking.

[b] Angle = arc sin $\sqrt{\text{percent}}$.

During the 1960-61 storage season, Stayman apples were dipped in solutions of DPA and Stop Scald (Santoquin) as indicated in Table 1. In addition, Stop Scald at 2000 ppm was sprayed on trees the day before harvest. Although the incidence of scald developed on the fruit during this storage season was considerably less than usual, differences between treatments were observed. Although Stop Scald, in comparison with the untreated check treatment, was effective in reducing the amount of scald development, its application as a liquid spray to trees 1 day before harvest proved less effective (5% level) than where the same concentration was used as a dip. Stop Scald at 2000 and 3000 ppm (2 and 3 parts of emulsion per 100 gallons) was equally as effective as 2000 ppm DPA.

During the 1961-62 storage season, Stop Scald and DPA were tested as dips on Stayman, Red Delicious, Paragon, and Red Rome apples from the first pickings. In addition, Stayman apples from the same pickings were placed in refrigerated storage and held for 4 days before treatments were applied. Scald records (Table 4) show that although Stop Scald and DPA reduced scald on all varieties tested, they were most effective on Stayman and Rome apples. On these varieties, 2 pints of Stop Scald per 100 gallons did very well, especially since these apples were harvested early and the Stayman were poorly colored and therefore unusually susceptible to scald. Stop Scald made a poor showing when used on cold apples (32° F). The temperature of the solution, used in an instantaneous dip, had no effect, however, on the performance of the dip. In this experiment, injury was observed on Rome and on Stayman apples treated at orchard temperature with 2000 ppm DPA.

Stop Scald and DPA were also effective in reducing Stayman spot. In this test 3 pints of Stop Scald was significantly better at the 1% level than 2 pints, and control was not as effective where cold fruit was treated.

DISCUSSION

The data presented show that both DPA and Santoquin are very effective in preventing scald and Stayman spot on Stayman apples, and scald on Rome apples when they are poorly used. These two anti-scald chemicals demonstrated less efficiency, however, in preventing this malady on Red Delicious and Paragon apples. Although DPA has demonstrated a real value in preventing scald from developing on stored fruit, it cannot always be used with safety on Stayman or Rome apples. Santoquin (Stop Scald) at 2 and 3 pints gave scald control equal to DPA in New Jersey experiments. In addition, injury from Santoquin has not been observed on any apple varieties in our tests. In this connection Smock (6) reports that some stain from the use of Stop Scald on Greening and Cortland apples was observed in his tests. This condition has not, however, been observed in New Jersey.

The experiment reported here demonstrates the importance of the temperature of fruit at time of treatment in determining the efficiency of the treatment. Santoquin was very effective when used as a dip for Stayman apples at orchard temperature. This efficiency was sharply reduced, however, when comparable apples were 32° F at the time of treatment.

DPA impregnated wraps and shredded paper were very effective against scald and Stayman spot on Stayman apples. Injury has not been observed from the use of DPA treated paper.

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REDUCTION OF ASTER YELLOWS IN FLAX VIA SYSTEMIC INSECTICIDES[1]

R. A. Frederiksen[2]

Summary

Two- to six-pound applications of phorate and a 2-pound application of Di-Syston, per acre, to farmers' fields reduced the incidence of six-spotted leafhoppers and thereby partially controlled aster yellows virus in flax. In artificially inoculated plots, band applications of phorate as low as 0.5 pound per acre significantly reduced the incidence of aster yellows. Similarly, 2 pounds of phorate per 100 pounds of seed reduced leafhopper populations sufficiently to control aster yellows virus partially. One and one-half pounds of phorate per acre caused some stand reduction and firing of leaves at St. Paul.

INTRODUCTION

Aster yellows can cause serious losses to the flax crop (1, 2). Possible control measures for this disease appear to be limited to the development of varieties resistant to the virus or control of the insect vector, Macrosteles fascifrons (Stål) (six-spotted leafhopper), or by use of systemic insecticides. Systemic insecticides have been used to control aster yellows in potatoes (4) and, more recently, experimentally in lettuce (3), by reducing the number of vectors. Since 1958 systemic insecticides have been tested in experimental plots and farmers' fields in Minnesota for control of the six-spotted leafhopper and aster yellows.

MATERIALS AND METHODS

Phorate and O, O-diethyl S-2-(ethylthio)ethyl phosphorodithioate (Di-Syston) were applied to farmers' fields with fertilizer spreaders or with a cyclone grass seeder.

Two fields of low-lime peat were selected for these tests at Bagley, Minnesota in 1958. A 10% granular formulation of phorate was applied with a fertilizer spreader at 6 pounds of active ingredient per acre and worked into the soil 1 day before sowing.

In 1959, similar applications of 2 and 4 pounds of phorate and 2 pounds of Di-Syston were made at Northome, Minnesota on sandy loam. All plots were nearly 1/2 acre in size and replicated three times. The prevalence of plants with aster yellows among 300 plants per plot and the number of leafhoppers, per 50 sweeps, in the areas that had been treated with insecticides were compared with the prevalence of aster yellows and number of insects in non-treated areas during the growing season. Yields were determined by harvesting 6 square yards per plot.

In experimental plots band applications of phorate were made by drilling the pre-weighed 10% granular formulation into rows 1.5 cm below the seed. For the seed treatment method, a formulation containing 44% active phorate in a carbon base was coated on the seed by means of a methylcellulose slurry. The band applications were 0.5, 1.0, and 1.5 pounds of active phorate per acre, whereas the seed was treated with 0.5, 1.0, and 1.5 pounds of phorate per 100 pounds.

The experiment was a split-plot design with treatments randomly alloted to whole plots except that the three treatments involving band applications and one control were grouped together in one randomly selected half of each replicate, and the three seed treatments and another control were allotted to the other half. In the analysis of variance the whole plots were treated as completely randomized. Sub-plots were three varieties, Arny, Marine, and Redwing, each seeded at a rate of 100 seeds per 6-foot row. Varieties were seeded 1 foot apart whereas whole plots were separated by 4 feet. The experiment was replicated four times.

Insects were made viruliferous by permitting them to feed on AYV-(aster yellows virus) infected aster or Nicotiana rustica plants for 5 days and transferring them to oats for 15 additional days for completion of the virus incubation period. Generally, 25 to 30% of the insects handled in this manner transmitted AYV. One-hundred viruliferous leafhoppers were introduced into cages (100 x 60 x 75 cm) which were placed over one-half of each plot in two replicates, 3 weeks after seeding. The other two replicates were inoculated similarly 2 weeks later.

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[1] Paper No. 4884, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, Minnesota.

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Cages were removed 3 weeks after the insects were introduced. Since there were no statistically significant differences between the two dates of inoculation, all data presented are the means of four replicates.

In the latter studies, notes were taken on stand, insect kill, phytotoxicity of the insecticide, and percentage of flax plants with aster yellows infection.

RESULTS

Phorate treated plots on both farms in 1958 had consistently fewer insects (Table 1). As a result, the average incidence of aster yellows was reduced from 7.5 to 1.5%. Similarly, in 1959 both rates of phorate markedly reduced the number of leafhoppers and percentage of plants with aster yellows (Tables 2 and 3). Di-Syston was less effective than phorate in controlling the insect vector and the incidence of aster yellows was greater in these plots.

Table 1. Periodic counts of M. fascifrons in 1/2-acre flax plots from two fields treated with 6 pounds of phorate per acre for insect control at Bagley, Minnesota, 1958.

Insects collected on:	Average number of leafhoppers per 50 sweeps[a]			
	Swenson Farm		Darst Farm	
	phorate	control	phorate	control
June 15:				
Adults	0.3	1.7	0.0	0.0
Nymphs	0.0	0.0	0.0	0.0
July 12:				
Adults	7.1	51.3	8.7	22.7
Nymphs	0.0	0.7	0.0	0.0
August 5:				
Adults	22.0	57.0	13.0	38.3
Nymphs	0.0	12.0	0.0	26.7

[a] Average of three replications, data supplied by A. Peterson, Department of Entomology, Fisheries, and Wildlife, University of Minnesota.

Table 2. Periodic counts of M. fascifrons present in 1/2-acre flax plots treated with two systemic insecticides at Northome, Minnesota, 1959.

Insects collected on:	Average number of leafhoppers per 50 sweeps[a]			
	Phorate		Di-Syston	
	4 lb/acre	2 lb/acre	(2 lb/acre)	Control
June 12:				
Adults	28.5	43.5	104.0	61.5
Nymphs	0.0	0.0	0.0	0.0
June 26:				
Adults	24.3*	33.3*	123.3	98.3
Nymphs	0.0	0.0	1.8	5.0
July 24:				
Adults	71.8*	78.5*	176.0*	348.3
Nymphs	9.3*	27.5*	115.0*	685.8

[a] Average of three replications, data supplied by A. Peterson, Department of Entomology, Fisheries, and Wildlife, University of Minnesota.

*Significantly lower than in the control (P equals 0.95). On July 24 the two phorate treatments were also significantly lower than the Di-Syston treatment.

Table 3. Percentage of aster yellows and yield of flax in 1/2-acre plots treated with systemic insecticides for the control of M. fascifrons at Northome, Minnesota, 1959.

Insecticide applications	% plants infected with aster yellows[a]	Yield/acre (bushels)
Phorate:		
4 lb/acre	4.8[b] a	14.5[c]
2 lb/acre	9.0 a b	16.9
Di-Syston:		
2 lb/acre	11.9 a b	17.0
Control	18.8 b	12.6

[a] Average infection among 300 plants selected in each of three replications.

[b] Means not followed by the same letter were significantly different at the 5% level (Student-Newman-Kuels' test).

[c] No significant difference.

Table 4. Percentage of flax plants in field plots with aster yellows after treatment with phorate for vector control[a].

Phorate applications	% plants with virus[d]
Band applications in lb/acre[b]	
1.5	15 f[e]
1.0	12 f
0.5	16 f
none	63 g h
Seed treatment in lb/100 lb of flax seed[c]	
1.5	44 f g
1.0	53 f g
0.5	89 h
none	86 h

[a] One-hundred viruliferous insects introduced in cages placed in each plot.

[b] Phorate applied to the soil in a strip directly below the flax.

[c] Phorate applied to the seed in a slurry of methylcellulose.

[d] Average of four replicates.

[e] Treatments not followed by the same letter were significantly different at the 1% level (Student-Newman-Kuels' test).

No differences in yield were detected in 1958; however, in 1959 all treated plots yielded higher than the control (Table 3). Although Di-Syston was less effective against the leafhopper, soil treated with Di-Syston produced the highest yield, indicating that high rates of phorate may be phytotoxic.

In 1960 stands were reduced nearly 15% in the 1.5-pound per acre phorate treatment, but no appreciable reduction in stand due to any of the other treatments. In the high-phorate treatment, a slight firing of lower leaves appeared 2 to 4 weeks after seeding, but subsequent growth did not appear to be damaged.

Insects in cages over plots treated with the band application of phorate were all killed within 2 days after their introduction, whereas there appeared to be little or no insect mortality in plots with seed treatment of phorate or the control plots.

Table 5. Percentage of flax plants in field plots with aster yellows after treatment with phorate for vector control in 1961[a].

Phorate applications	% plants with virus[b]
Band application in lb/acre[c]	
0.2	42.9 f[d]
0.5	10.9 g
1.0	4.6 g
Seed treatment in lb/100 lb flax seed[e]	
1.5	23.2 g
2.0	12.7 g
Control	57.8 f

[a] 50 viruliferous leafhoppers introduced in cages placed in each plot.

[b] Average of two replicates.

[c] Phorate was placed in the soil in a strip directly below the flax.

[d] Treatments not followed by the same letter were significantly different at the 5% level (Student-Newman-Kuels' test).

[e] Phorate applied to the seed in a slurry of methylcellulose.

Significant reductions in the percentage of aster yellows infection were obtained as a result of all three band applications of phorate. AYV was controlled somewhat by treating the seed with 1.5 pounds of phorate per 100 pounds of seed (Table 4). This insecticide was available in the plant as long as 5 weeks following seeding. At the plant population used, rate for rate, the dosage used in the band application was about 10 times as great as the seed treatment on an individual-plant basis. Therefore, 1.5 pounds of phorate per 100 pounds of seed equals, on an average, one-third the dosage of the 0.5-pound per acre band treatment. On the basis of this test, the most efficient level of insecticide may lie somewhere near 1/2 pound per acre for band applications or perhaps 2 to 4 pounds of insecticide per 100 pounds of seed.

Surprisingly, there was a definite difference in the incidence of virus in the flax varieties compared. Redwing had significantly fewer virus-diseased plants than did Arny or Marine.

The utility of systemic insecticides for AYV control was repeated during 1961 in an experiment similar to the one done in 1960. In 1961 the rates used were 0.2, 0.5, and 1.0 pound of phorate for the band application and 1.5 and 2.0 pounds of phorate per 100 pounds of seed with the phorate applied to the seed in a methylcellulose slurry.

Because of poor leafhopper survival in the greenhouse during April and May, only two replicates of this experiment were inoculated with viruliferous leafhoppers. Nevertheless, control of AYV by reducing populations of leafhoppers with phorate was excellent (Table 5). Nearly 60% of the control plants had AYV, while less than 5% were infected in plots in which phorate was applied at 1 pound per acre.

DISCUSSION

The development of flax varieties highly resistant to aster yellows will require a long and extensive breeding program. Systemic insecticides therefore may provide an adequate control of this disease until other methods are devised.

Partial control of both insects and virus has been achieved by broadcast application of systemic insecticides. However, from a cost standpoint, this method is not acceptable because of the quantity of insecticide needed.

Low rates of phorate, which were not phytotoxic, were effective in controlling leafhoppers for up to 5 weeks after application, and possibly provide partial protection even longer. A 5-week protection period would probably control the early-arriving migrant leafhoppers during the latter part of April and the first weeks in May, a more or less typical situation in Minnesota. This is especially significant because these leafhoppers are usually more viruliferous than their progeny. Control of the early-arriving leafhoppers should delay virus infection and thus lessen the damage to the flax yield.

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ASCOCHYTA LEAF SPOT FOUND ON THREE WINTER OAT SELECTIONS IN KENTUCKY

H. Randolph Richards[1]

In early April 1962, a general yellowing of certain plots was noted in the Uniform Northern Winter Oat Nursery on the Western Kentucky Experiment Substation farm at Princeton. The plots so affected were the experimental lines Kentucky 56-527 (C. I. 7483), Kentucky 56-518 (C. I. 7484), and Missouri 0-4831 (C. I. 7495). Closer examination revealed necrotic spots associated with the tip yellowing of the leaves, with easily observable pycnidia scattered throughout the spots.

Under the microscope, each pycnidium possessed an ostiole and contained numerous 2-celled, oblong, hyaline conidia. Reference to Barnett (1) led to placing the causal organism in the genus *Ascochyta*. More detailed diagnosis by E. M. Johnson and Lawrence Henson, plant pathologists at the University of Kentucky, Lexington, confirmed the preliminary identification.

By April 16, 1962, the affected plots showed no evidence of any injury from the disease except for partly killed leaves around the base of the plants. No significant damage from the disease is anticipated.

According to Simons and Murphy (2), this disease is most prevalent in the Pacific Northwest, and they made no mention of its having been reported in the Southeast. Poehlman[2] stated that neither he nor T. D. Wyllie had observed *Ascochyta* on oats in Missouri. However, he added that Wyllie found that the disease had been reported from Georgia (3).

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[1] Assistant Professor of Agronomy, University of Kentucky, Western Kentucky Experiment Substation, Princeton.

[2] Poehlman, J. M. 1962. University of Missouri. Correspondence with the author.

OCCURRENCE OF THE BEAN ROOT ROT FUNGUS, FUSARIUM SOLANI F. PHASEOLI, IN HAWAII[1]

E. E. Trujillo[2]

Fusarium solani (Mart.) Appel & Wr. f. *phaseoli* (Burk.) Snyd. & Hans. has never been reported from Hawaii. The pathogen was found for the first time in 1962, in the Kula district, Maui, Hawaii, causing a dry cortical stem rot on the bean variety Hawaiian Wonder. The fungus was isolated from the rotted bean tissues on acidified potato-dextrose agar and the identification was based on cultural and morphological characters of the macroconidia when grown on PDA (2, 3).

Bean seedlings var. Hawaiian Wonder were inoculated with monosporic cultures of *F. solani* f. *phaseoli* and *Fusarium solani* species. A spore suspension of the inoculum was placed with a micro dropper on the hypocotyl of the beans grown aseptically on water agar in deep Petri dishes. A week later, typical reddish-brown lesions were visible in all bean hypocotyls inoculated with the *F. solani* f. *phaseoli* obtained from Maui, whereas all beans inoculated with a soil isolate of *F. solani* species showed no lesions. Extensive bean root rot also was produced on beans grown in autoclaved soil containing a spore suspension of the Maui isolate.

In all probability the original introduction of *F. solani* f. *phaseoli* in the State of Hawaii occurred years ago, when bean seeds were brought to Hawaii from mainland areas where the fungus was already present. Since the fungus is soil-borne as thick-walled chlamydospores (1), it probably was introduced with soil particles on the surface of bean seeds.

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[1] Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Miscellaneous Paper No. 153.

[2] Assistant Plant Pathologist, University of Hawaii, Kauai Branch Station, Kapaa, Kauai.

CORRECTIONS

REPORTER, May 1961 issue (Volume *45*, Number 5), page 377. Table 2, Trial 4, change the percentage soil rot for Norwich U587 to read: *11.3*, instead of 10.8; in the same column, LSD .05 should read: *8.42*, instead of 3.45. Page 378, Table 2 (continued), Trial 5, change the percentage soil rot for Nabac 25 + captan to read: *7.9*, instead of 8.9; in the same column, LSD .05 should read: *5.81*, instead of 4.87. In both trials 4 and 5 the percentages of soil rot were based on three replications, not five.

REPORTER, September 1962 issue (Volume *46*, Number 9), page 682. In the fourth paragraph, change lines 7 and 8 to read: specimens of *Q. falcata* var. *pagodaefolia* *shows* extreme susceptibility (Fig. 1) while the other *is* highly resistant, ...

The Reporter regrets the error in the tense of the verbs.

THE PLANT DISEASE REPORTER

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 46 Number 12

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Growth Through Agricultural Progress

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CONTENTS

1. Preliminary studies for estimating the disease potential of Phytophthora parasitica var. nicotianae in infested tobacco soils
SAMUEL F. JENKINS, Jr. 825

2. Cycloheximide and the control of hawthorn leaf blight under nursery conditions
ROBERT E. INMAN 827

3. Sudden decline of peach trees in Georgia
W. A. CHANDLER, et al. 831

4. Loss in oats grown for forage in Texas due to occurrence of new races of crown rust
LUCAS REYES and MAURICE C. FUTRELL 835

5. The occurrence of ergot on smooth bromegrass in Michigan
DELBERT L. BIERLEIN 838

6. The role of Pratylenchus penetrans in the development of strawberry black root rot
TSEH-AN CHEN and AVERY E. RICH 839

7. Determination of dinitro-ortho-cresol in apple
K. G. PARKER, et al. 844

8. Effects of light, temperature, and fertilizer on infection of wheat leaves by Septoria tritici
HURLEY FELLOWS 846

9. Cucumber mosaic virus on safflower
J. M. KLISIEWICZ 849

10. A new greenhouse technique for evaluating fungicides for control of cotton sore-shin
HANY M. ELSAID and J. B. SINCLAIR 852

11. American dagger nematode (Xiphinema americanum) on coffee in Guatemala, with suggestions for nematode control in nurseries
GERALD THORNE and EUGENIO SCHIEBER 857

12. Nematodes associated with wild yam, Dioscorea sp., with special reference to the pathogenicity of Meloidogyne incognita incognita
W. R. JENKINS and G. W. BIRD 858

13. Reduction of postharvest brown rot and Rhizopus decay of eastern peaches with hot water
W. L. SMITH, Jr. 861

14. Physiologic specialization of Puccinia graminis avenae in Colombia
JUAN ORJUELA, et al. 866

15. Nematophagus fungi recovered around Highlands, North Carolina
WILLIAM A. FEDER 872

16. A new Phytophthora fruit rot of Annona squamosa from India
VASANT GURUNATH RAO, et al. 874

17. Differentiation of tobacco ringspot and tomato ringspot viruses by experimental host reactions
D. M. McLEAN 877

18. Symptoms of tobacco ringspot virus on papaya
D. M. McLEAN and E. O. OLSON 882

19. Gummy stem blight resistance of introduced watermelons
GROVER SOWELL, Jr. and GERALD R. POINTER 883

20. Effect of liming of soil on barley powdery mildew
J. VLAMIS and C. E. YARWOOD 886

21. Aspergillus crown rot of peanut in Georgia
CURTIS R. JACKSON 888

22. Occurrence of two bacterial diseases on new hosts in Israel
ZAFRIRA VOLCANI 893

23. Unusual case of delayed foliation and dieback of Moneymaker pecan
J. R. COLE and J. H. HUNTER 894

PRELIMINARY STUDIES FOR ESTIMATING THE DISEASE POTENTIAL OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE IN INFESTED TOBACCO SOILS[1]

Samuel F. Jenkins, Jr.[2]

Abstract

A laboratory method of estimating the disease potential index (DPI) of P. parasitica var. nicotianae in soils was demonstrated. This method consisted of making serial dilutions of infested soil with sterile soil, adding a known amount of water and two tobacco leaves as indicators. The highest DPI in infested soil was 4096 and the lowest was 16. The DPI was 0 for soils in which there was no known infestation of black shank. The use of this method should aid in the development of control measures for the disease.

Phytophthora parasitica Dast. var. nicotianae (Breda de Haan) Tucker, the causal agent of the black shank disease of tobacco, has become an increasingly serious problem in the flue-cured tobacco belt of Georgia (1, 2). Although black shank-resistant varieties are available, the majority of Georgia growers prefer the susceptible varieties. A quick laboratory method for the quantitative estimation of the disease potential of black shank infested soil would be highly desirable in the study of various control methods such as rotations and chemical treatment.

The disease potential of certain Phytophthora species has been evaluated by several methods. Nusbaum, et al. (4) found in preliminary tests that soils naturally infested with P. parasitica var. nicotianae could be diluted up to 100 times with sterile sand and still contain sufficient inoculum to kill susceptible plants in 20 days. The fungus may also be detected by placing leaves of susceptible tobacco in soil suspensions (7). Klotz and DeWolf (3) used lemon fruit to trap P. citrophthora (R. E. Sm. & E. H. Sm.) Leonian, P. parasitica Dast. and P. syringae Kleb. in soil suspension. Tsao (5, 6), using lemon fruit as an indicator of disease development, devised a serial soil dilution method to estimate the disease potential of P. citrophthora and P. parasitica.

The objective of this study was to develop a serial dilution end-point method for a quantitative estimation of the inoculum potential of P. parasitica var. nicotianae in naturally infested soils.

MATERIALS AND METHODS

Samples of black shank infested soil were obtained from three locations in North Carolina[3] (Edgecombe, Pitt, and Lenoir counties) and two in Georgia (Tift and Mitchell counties). The North Carolina samples were taken from three black shank disease nurseries just prior to planting and the Georgia samples came from fields where black shank was actively developing on growing tobacco. Three samples were taken from each of the Georgia fields. Sample 1 was taken from an area where 90% or more of the tobacco was infected with black shank, sample 2 was taken from an area where less than 25% of the tobacco was infected, and sample 3 was taken from an area where plants were expressing no visible symptoms of black shank. The method of dilution of the soil has been described by Tsao (5, 6). Only slight modifications were made in that a taller cup (No. 16 Lily) was used to support the tobacco leaves which were used as an indicator of the presence of the fungus. Two leaves of the tobacco variety Broadleaf Hicks were placed with the petiole end into the soil suspension. The infested soil was diluted with an autoclaved soil from the same sample. Dilutions were 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096 and 1/8192. Disease ratings were made by both observation of visual symptoms and microscopic examination of leaf tissue.

A parallel experiment involved planting a susceptible variety seedling (Variety 402) in 150 cc of soil with the same dilution series as in the laboratory cup method. Disease ratings were based on visual symptoms of disease expression and microscopic examination of stem tissue.

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The author wishes to express his appreciation to B. A. Golden, Technical Assistant, for his contributions to this investigation.

[2] Assistant Plant Pathologist, Georgia Coastal Plain Experiment Station, Tifton. University of Georgia, College of Agriculture Experiment Stations.

[3] Acknowledgment is made to Drs. E. L. Moore, N. T. Powell, and P. D. Dukes for assistance in obtaining the North Carolina soil samples.

RESULTS AND DISCUSSION

A dilution end point was detected in each of the infested soils tested. The lowest end points were in the soil from Lenoir County, North Carolina, and Tift County, Georgia, and the highest from Mitchell County, Georgia. The Mitchell County sample was from a field where the fungus was actively parasitizing growing tobacco. In the field from which the Mitchell County sample was obtained, approximately 90% of the tobacco had black shank and the inoculum potential was probably near its peak. The disease potential index (DPI) as used by Tsao was the reciprocal of the highest dilution in which a positive infection took place. The DPI of the black shank soils tested are given in Table 1.

Where there was no apparent black shank activity the DPI was 0 in the soils tested. The laboratory method was less time- and labor-consuming and the DPI was reached more quickly than by growing susceptible plants in the cups.

Table 1. The disease potential index (DPI) of soils tested for inoculum potential of the black shank fungus.

Soil location	Sample	DPI	
		Laboratory	Greenhouse
Mitchell County, Ga.	1[a]	4096	2048
	2[b]	128	128
	3[c]	0	0
Tift County, Ga.	1[a]	32	32
	2[b]	16	16
	3[c]	0	0
Edgecombe County, N. C.	1[d]	32	32
Lenoir County, N. C.	1[d]	16	16
Pitt County, N. C.	1[d]	32	16

[a] From area of high infestation (tobacco 90% or more infected).

[b] From area of low infestation (tobacco 25% or less infected).

[c] From area of no known infestation.

[d] From area where black shank nursery is maintained but tobacco was not growing at time of sampling.

This method of detecting the DPI of black shank should prove useful in developing control methods for the disease other than resistant varieties. In control methods such as rotations and chemical treatments it should be possible to detect changes in the disease potential due to such practices. Further work is being conducted on using this method for estimating the inoculum level in pond water and assessing the value of the method for laboratory screening of chemicals to be used for field and chemical control experiments.

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CYCLOHEXIMIDE AND THE CONTROL OF HAWTHORN LEAF BLIGHT UNDER NURSERY CONDITIONS[1]

Robert E. Inman[2]

Abstract

Studies were conducted to determine the proper time of application of minimal amounts of cycloheximide needed to control hawthorn leaf blight under conditions of high inoculum intensity provided by natural infection of trees in nursery blocks. Satisfactory disease control was obtained with single sprays of 1 ppm or 5 ppm cycloheximide, and with repeated sprays of dodine and zineb fungicides. The concentration of cycloheximide was critical in regard to the extent of disease development at the time of application. A higher concentration of cycloheximide was required to eradicate established infections than to prevent new infections. A single spray of 5 ppm cycloheximide applied during initial defoliation prevented further defoliation for the remainder of the season.

INTRODUCTION

Hawthorn leaf blight, caused by the ascomycetous fungus Fabraea maculata Atk., and perhaps the most spectacular of the more common leaf spot diseases of trees, is of serious concern to homeowners and nurserymen in the central and eastern States. The Paul's Scarlet variety of English hawthorn, a long-time favorite of homeowners, is especially susceptible. Infected trees are quite regularly defoliated by mid-September (3).

In the past years control has been achieved with sprays of ferbam or thiram fungicides (1), or with phenyl mercury fungicides such as Phix or Puratized Agricultural Spray (2), applied at 10-day intervals. These recommendations have adequately met the needs of homeowners, who generally have no more than a few scattered trees to maintain.

The nurseryman, however, thinks in terms of hundreds of trees. Furthermore, the convenience of his operation requires that these trees be planted closely together, frequently in a large block. These two conditions -- a large number of uniformly susceptible plants growing in a tightly confined area -- contribute considerably to a rapid buildup of inoculum and a subsequently high level of disease intensity. In spite of this disease problem, a disease control program involving spray applications every 10 days is usually not practical in the large nursery.

A series of spraying experiments was conducted under nursery conditions over a 2-year period to determine 1) the material giving the highest degree of disease control with a single or a limited number of applications, and 2) the time at which this material could be most advantageously applied. The antibiotic fungicide cycloheximide was included in the tests because of the findings by Strong (3) that 20, 10, or 5 ppm cycloheximide applied three times during July resulted in excellent disease control.

MATERIALS AND METHODS

Tests were conducted during the summers of 1961 and 1962 at the Weston Nurseries, Hopkinton, Massachusetts on 6-year-old trees of Paul's Scarlet variety of English hawthorn, Crataegus oxycantha var. pauli, or the double pink variety rosea-plena. The preliminary studies of 1961 involved the use of three fungicides: cycloheximide (Actispray)[3] 5 ppm, dodine (Cyprex) 1/4 lb/100 gal., and zineb (Parzate) 2 lb/100 gal. Each material was applied one, two, and three times during June and early July to duplicate plots of 7 to 10 trees each with a hydraulic sprayer at 400 psi pressure. Single-spray treatments were applied on July 11, a time of high disease intensity, to determine their effect in regard to the prevention of defoliation. Applications for 2-spray treatments were made on June 20 and July 11, and for 3-spray treatments on June 6, June 20, and July 11. The effects of treatments were estimated by disease index ratings. Disease was due to natural infection.

In accordance with the indications of 1961 tests, more definitive studies were conducted on Paul's Scarlet variety in 1962 to determine the effects of single-spray treatments. Cycloheximide was applied to trees in four separate plots on June 19 and July 6 at 5 ppm and 1 ppm concentrations. Additional plots were sprayed on June 19 and July 6 with dodine, 1/4 lb/100 gal., and used as treated controls. A non-sprayed plot was also included as a general control. Eight trees comprised each plot. Ten leaves from each of the middle six trees in each plot were collected on July 30, and again on August 31. The number of leaf-spot lesions in each 10-leaf sample was determined. Data were subjected to an analysis of variance.

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[3] Cycloheximide supplied by the The Upjohn Company, Kalamazoo, Michigan.

RESULTS AND CONCLUSIONS

The results of the 1961 tests indicated that repeated applications of all fungicides tested would satisfactorily control the disease under nursery conditions. Cycloheximide, however, showed exceptional control in relation to the other two materials, indicating that it might [illegible] fective in a limited spray program. Dodine appeared to offer a higher degree of disease control than did zineb. Neither of these two materials, however, showed promise for use on a limited scale in the nursery.

Figure 1 shows selected twig specimens representing the degree of control obtained with two sprays of the respective fungicides in preliminary tests. Photographs were taken 34 days after the last sprays were applied. By September 6, 57 days after the last spray, control trees were 90 to 95% defoliated. Trees sprayed two or three times with zineb suffered slight defoliation. No defoliation was observed in comparable plots sprayed with cycloheximide or dodine.

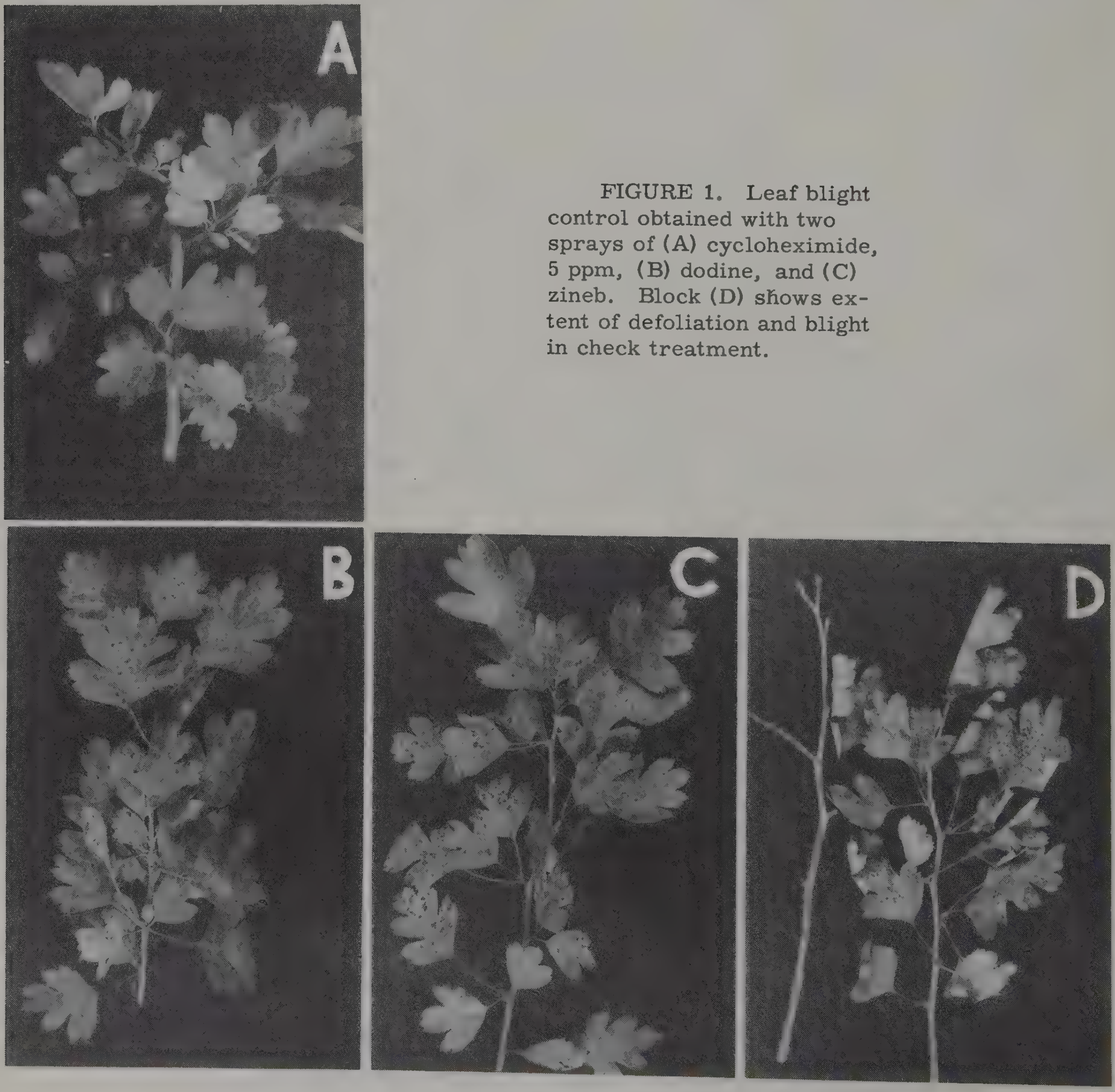

FIGURE 1. Leaf blight control obtained with two sprays of (A) cycloheximide, 5 ppm, (B) dodine, and (C) zineb. Block (D) shows extent of defoliation and blight in check treatment.

A single spray with cycloheximide applied on July 11, a time of severe disease intensity in control plots, completely precluded defoliation as of September 6 in the double pink variety. Comparable plots treated with zineb or dodine prevented the excessive degree of defoliation observed in control plots but did not approach the degree of effectiveness demonstrated with cycloheximide.

Tests during the summer of 1962 were designed to more adequately define the capacity of a single-spray treatment with cycloheximide to control the leaf blight disease. Depending upon the date of application, lesion counts were made 41 or 24 days after treatment, and again 70 or 55 days after treatment (Table 1).

Table 1. Control of hawthorn leaf blight with single sprays of cycloheximide (Actispray), as indicated by numbers of lesions. Six 10-leaf samples collected on two dates following each treatment.

Treatment	Date of treatment	Total lesions in six 10-leaf samples			
		July 30	Mean$\sqrt{\ }$[a]	Aug. 31	Mean$\sqrt{\ }$[a]
Cycloheximide, 5 ppm	June 19	85	3.49	480	7.87
do.	July 6	392	7.47	341	7.36
Cycloheximide, 1 ppm	June 19	24	1.75	367	6.94
do.	July 6	1023	12.38	2913	21.05
Treated control (dodine)	June 19	191	5.53	1452	15.02
	July 6	2102	17.25	4610	27.61
Control	Not sprayed	3884	23.32	Defoliated	--
LSD (Mean$\sqrt{\ }$)[a] .05			7.17		5.66
.01			9.60		7.60

[a] Analysis of variance was determined using square roots of lesion counts in each 10-leaf sample. "Mean$\sqrt{\ }$" signifies treatment mean of the square roots of sample counts.

A single spraying with cycloheximide, either at 5 ppm or 1 ppm, will give satisfactory control in the nursery if the date of application is considered. Cycloheximide at 5 ppm gave satisfactory control whether applied as an early (June 19) spray or as a late (July 6) spray. Cycloheximide at 1 ppm provided a degree of disease control comparable to that given by 5 ppm if applied as an early spray. When applied as a late spray, however, 1 ppm cycloheximide provided a significantly lower degree of control than did 5 ppm.

The same trend was seen in the effectiveness of dodine, although this material would not be recommended for use in a single-spray program because of its relatively poor residual capacity.

The lesion counts recorded on July 30 from samples sprayed on July 6 indicate that the first surge of infection occurred shortly before the July 6 spraying. Only a small percentage of this infection, though established in the host tissue by July 6, had as yet developed sufficiently to cause lesions on this date.

In this respect it is interesting to compare the effects of the July 6 cycloheximide treatment at 5 ppm opposed to 1 ppm as observed on both recording dates. There was no significant difference observed on either recording date between dates of spraying in the 5 ppm treatments. In the 1 ppm treatments, however, significance at the 1% level was observed between spray dates on both recording dates. If the hypotheses made above concerning the time of the first surge of infection and the symptom development are correct, the greater degree of disease control achieved by the 5 ppm cycloheximide treatment on July 6 over that provided by 1 ppm would appear to be due to the greater capacity of the 5 ppm treatment to eradicate the infection established just prior to treatment. Even though significance was not observed between the effects of 5 ppm and 1 ppm until the later recording date, the trend was apparent on July 30. The fact that no significance was observed between June 19 cycloheximide treatments at either recording date indicates that both the 5 ppm and 1 ppm treatments were statistically equal in their effects regarding prevention of new infections.

On the assumptions made above, the conclusion is drawn that new infection may be prevented by lower concentrations of cycloheximide than required to eradicate established infections. The method by which new infection is prevented, however, is open to question. If a protective action of cycloheximide is effective prior to penetration, that is, during or before spore germination, the fungitoxicity in question is truly preventative. If, however, the fungitoxic mechanism is active only within the leaf tissue, prevention of new infection is technically a process of eradication, whereby penetrating hyphal elements are killed before infection is established in the host. In the latter case the protective capacity of varying doses of cycloheximide would appear to be a function of the amount of fungal tissue present in the leaf. More critical work must be conducted before the preventative mechanism can be defined.

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SUDDEN DECLINE OF PEACH TREES IN GEORGIA[1]

W. A. Chandler, J. H. Owen, and R. L. Livingston[2]

Summary

A rapidly developing disease of peach trees in Georgia in the early spring of 1962 resulted in the death of approximately 200, 000 trees. Trees died suddenly at or shortly after blooming. Affected trees showed a wilt of immature foliage and a brown discoloration of the cambial region on trunks and limbs, accompanied by a yellowish exudate from the bark and a characteristic "sour-sap" odor. Although yeasts and bacteria seemed responsible for fermentation of the bark, no pathogenic organism was consistently associated with the diseased tissue. The pattern of development indicated that physical factors probably were responsible for the disease. Excessive rainfall in late winter, resulting in waterlogged soils, and a freeze in February, following a period of unseasonably warm weather, were possible contributing factors.

INTRODUCTION

A sudden dying of peach trees occurred in Georgia peach orchards during the early spring of 1960 and of 1961. In 1962, the same condition began to appear shortly after the buds had started to open. Tree losses greatly exceeded those of the previous years and were generally distributed throughout the peach-growing area of south Georgia. Damage was less severe in orchards in the Georgia Piedmont. An estimated 200, 000 trees died during the spring and summer of 1962.

SYMPTOMS

Trees died chiefly during blossoming or shortly after the foliage buds opened, although some continued to die throughout the summer. The young foliage failed to mature and soon wilted (Fig. 1). Drops of yellow to orange exudate oozed from the bark at various places on the trunk and limbs (Fig. 2). Bark of affected trees had a characteristic "sour-sap" odor. When the bark was removed, the cambial tissue and outer wood showed a rusty-brown discoloration (Fig. 3). It was not uncommon to find a portion of a limb showing internal browning while the remainder of the tree appeared normal. Frequently the discoloration developed first at the crotch and progressed into the larger limbs. It often extended over most of the limbs and aboveground portions of the trunk but stopped at the ground level. The outer bark usually was slightly sunken and somewhat spongy over the affected area. In early spring, incisions revealed extensive development of internal browning in trees that had not yet developed external symptoms.

Trees in affected orchards had roots with greatly enlarged lenticels (Fig. 4), even near the surface of the soil. Moreover, root examinations frequently revealed a distinct absence of healthy feeder roots. In some orchards cankers appeared on limbs of affected trees; these were usually associated with pruning wounds. The bark cracked and sap excretions produced a black surface discoloration. Similar cankers appeared, however, on otherwise healthy trees.

The majority of trees affected had appeared sound at the end of the previous season. The disease developed suddenly in early spring, and large numbers of trees died within a short period. Most of the affected trees were in the same stage of decline, and sometimes large portions of an orchard were affected. Some trees that appeared to be injured early in the season recovered; others that showed no external symptoms in the spring died at a later date. The affected trees varied in age from newly-set trees to those 5 to 8 years old, with more decline among the older trees.

BIOTIC FACTORS

Isolations from the discolored wood and inner bark of trees in the earliest stages of decline frequently yielded no organisms. Several species of bacteria and yeasts were consistently

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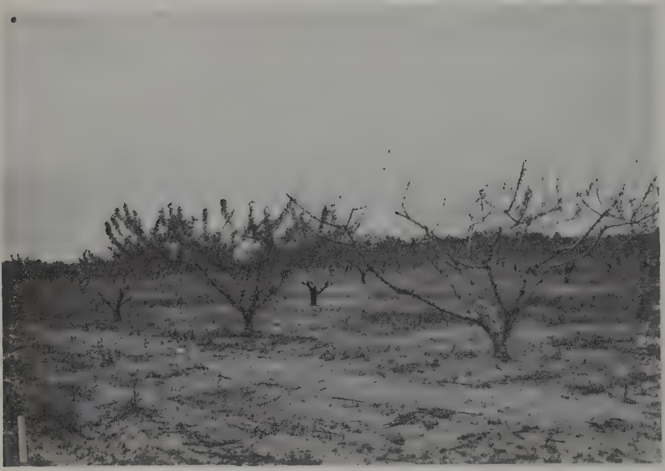

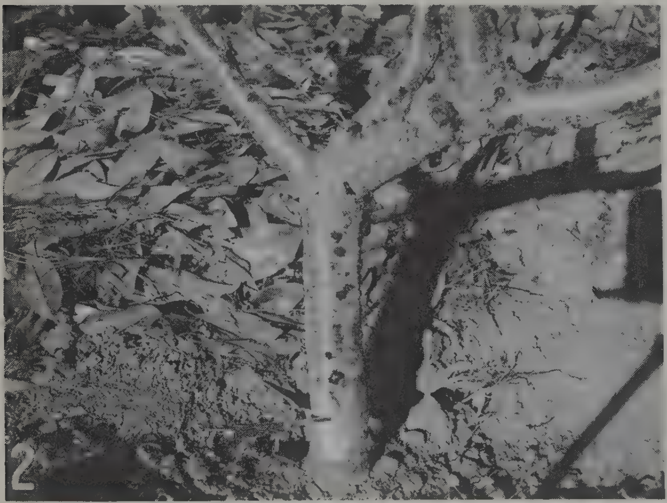

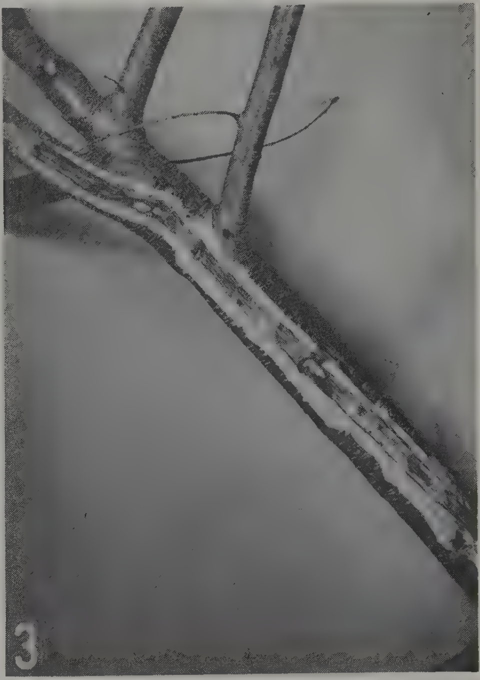

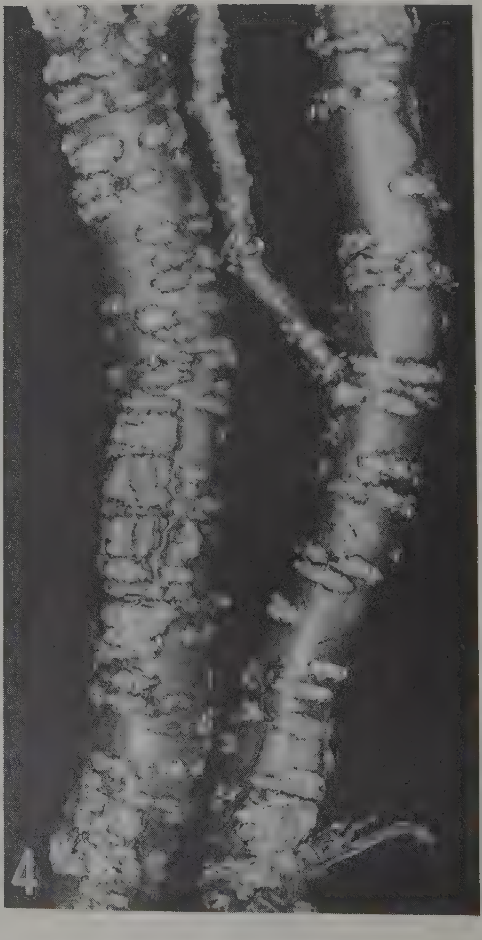

FIGURE 1. Peach orchard with most trees dead. Tree in right foreground in state of severe decline, while tree in left foreground appears healthy.

FIGURE 2. Three-year-old peach tree showing exudate from affected area of trunk and brown discoloration beneath the bark.

FIGURE 3. Brown discoloration of cambial region of young peach tree.

FIGURE 4. Enlarged lenticels on roots of affected peach tree.

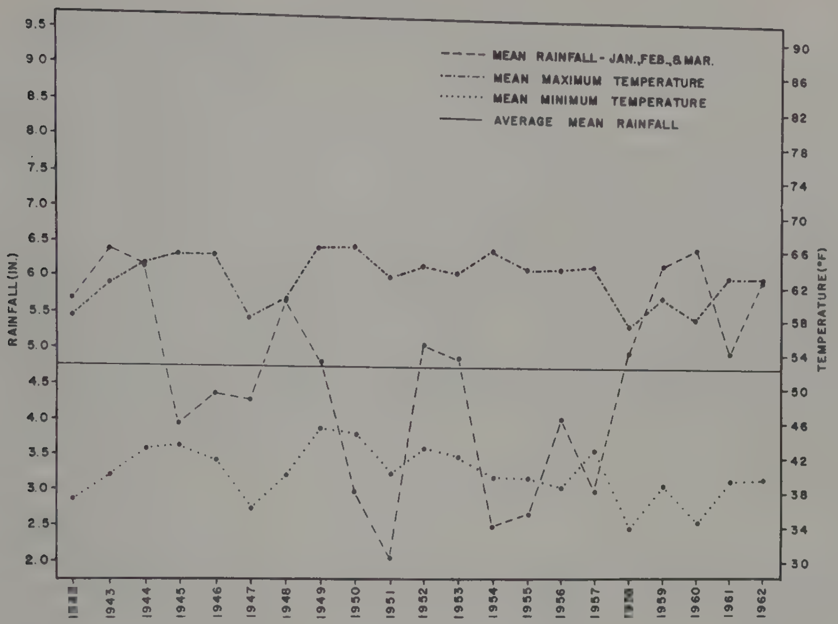

FIGURE 5. Graph of rainfall and temperature data for the January-March period at Fort Valley, Georgia, 1942-1962 (Prepared by C. D. Spivey).

present in bark that had developed the characteristic "sour sap" odor and exudate. Species of Sphaeropsis, Phoma, Macrophoma, Pestalotia, Cladosporium, and Cystospora were isolated from older lesions, but none of these was consistently associated with the discolored tissue. Isolations from soil failed to produce fungi such as Phytophthora spp. that might be considered responsible for the loss of feeder roots. Trees were frequently damaged by nematodes and borers, but this was true also of trees that showed no symptoms of decline. Inoculations were made on young peach trees with the isolates of bacteria and yeasts. After 5 months, no infection has been obtained.

PHYSICAL FACTORS

Weather data from Fort Valley, Georgia (Fig. 5) showed that rainfall during the first 3 months of each year from 1958 to 1962 was well above the 40-year mean, while no comparable period of successive wet seasons has occurred since the years 1942-1945. December 1961 rainfall was also unusually heavy. In most orchards dying trees occurred on low, poorly-drained sites. The condition was found also in orchards on sandy soils on slopes, but on many of these sites there was a hard pan close to the soil surface. Growers indicated that sudden decline occurred on soils that had been continuously wet for long periods during January-March in 1962, even in orchards on the lighter soils along ridges or slopes.

Weather data (Fig. 5) showed no exceptionally low temperatures during the January-March period of 1962. Nevertheless, the temperature pattern may be of significance. At Fort Valley there were over 800 hours at or below 45° F by January 21, 1962. This was enough to break dormancy in most of the varieties grown in that area. From January 22 to February 5 the weather was unseasonably warm, with maximum temperatures in the 70's for 9 days and in the 60's the other 6 days. The mean maximum temperature was 69. 5°. On February 6, 7, and 8 the minimum temperatures were 28°, 22°, and 28°. On February 9, the maximum was 79°. Injury to trees was first observed soon after this cold period.

At Experiment, Georgia, which is typical of the middle-Georgia peach belt, where far less injury occurred, maximum temperatures from January 22 to February 5 were in the 70's only 4 days and in the 60's 8 days, with 3 cooler days and a mean maximum of 64. 3° F. The minimum temperatures for February 6, 7, and 8 were 24°, 17°, and 32°. The lowest temperature was thus 5° below that at Fort Valley but followed a period of 15 days with maximum temperatures that averaged 5. 2° cooler than at Fort Valley.

DISCUSSION

Although the yeasts and bacteria present in the injured bark probably were responsible for the fermentation producing the yellowish exudate and "sour-sap" odor that were pronounced characteristics of the disease, no evidence that they were pathogenic was obtained. No other organisms were consistently associated with the affected bark, and in the incipient stages of injury the discolored tissue often proved to be sterile. The possibility that sudden decline might be caused by soil-inhabiting pathogens has not been completely eliminated. Nevertheless, the pattern of development suggests that this disease is noninfectious.

The disease developed rapidly, killing trees that were apparently healthy the previous fall. It appeared almost simultaneously on large numbers of trees in many orchards throughout the south Georgia area, and most of the affected trees were in the same stage of decline. The disease did not appear to spread within affected orchards after the initial severe loss in the spring, and trees that continued to die during the summer seemed to have been injured earlier. This history suggests that physical factors in the environment were responsible.

The possibility that sudden decline was caused by excessive soil moisture is supported by its occurrence on poorly drained sites, the high rainfall during late winter which resulted in saturation of the soil for long periods, and the presence of hypertrophied lenticels on the roots. Stem injury to hemlock caused by excessive soil moisture (1) offers a parallel case. Baldwin and Elliott (1) reported that trees having cankered areas in lower trunk region and splitting of bark recovered when moved to better sites during early stages of the disease but died if not moved. Some of the poorly drained sites were on slopes. This hypothesis could be tested more adequately if records of peach tree mortality in past years were available for comparison with weather records. Kienholz (7) reported that pear trees survived and produced a crop for three seasons with flooding for at least 3 months annually, whereas a single row of apricot trees died within 2 weeks of the start of flooding the first year. The author states "This behavior of the apricot trees is in accord with the widely recognized susceptibility of species of Prunus to waterlogging of the soil."

Temperature records rule out winter killing of dormant trees as a factor. Campbell and Hadle (2) found that 33 peach varieties survived -29° F with varying injury but no trees were killed. Cold injury to the active cambium, however, is a possibility deserving further exploration. Relatively moderate cold might cause such injury when it follows warm periods long enough to initiate cambial activity, as in the Fort Valley area in 1962. In 1960 a similar period of warm weather and heavy rainfall in January and February was followed by a freeze in early March and numerous dying trees were noticed shortly afterward. Donoho and Walker (4) found that when small peach trees in containers were exposed to 40° then moved to 65° for 7 days their hardiness at 5° was significantly reduced. They also found injury in trees subjected to freezing temperatures as moderate as 21°. Vasilyev (8) concluded that waterlogged plants are more susceptible to cold injury and that further waterlogging increases the injury caused by cold. Earlier work in Georgia (3, 6) showed that cold injury was greatly reduced by the application of complete fertilizers containing at least 0.48 pounds of nitrogen per tree. Many of the orchards affected in 1962 received far less nitrogen. Sudden decline, therefore, may have resulted from a complex of factors, and their effects may have been intensified by the shift in recent years to less vigorous early varieties of peaches (5).

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LOSS IN OATS GROWN FOR FORAGE IN TEXAS DUE TO OCCURRENCE OF NEW RACES OF CROWN RUST[1]

Lucas Reyes and Maurice C. Futrell[2]

Summary

Forage production of seven oat varieties was studied for 3 years under heavy natural crown rust epidemics at Beeville, Texas. Rust-susceptible varieties produced less forage than rust-resistant ones. The rust caused the heaviest damage near the end of the growing season in April or early May. In some years the grazing season has been cut short by 1 month to 6 weeks because of crown rust damage. The occurrence of new races of crown rust sharply reduced the pounds of air-dry forage produced by oats. During the first two growing seasons of this study, the 'Moregrain' oat variety produced more forage than the 'Suregrain' variety, but during the last season a 30% moderately susceptible type pustule crown rust infection developed on Moregrain and this variety produced 916 pounds less forage per acre than did Suregrain.

INTRODUCTION

Studies have been conducted in Texas on the reduction of grain yield by rusts (2) but no studies have been conducted on the reduction of forage yields caused by rust. Oats are grown widely for livestock pasture in southern Texas. Recent published data (4) place this acreage in excess of one million acres. Diseases play a major role in the amount of forage that each acre will produce. Rusts, *Helminthosporium*, and virus diseases are among the major ones that cause losses of pasture forage in Texas.

During the early years of growing oats for pasture in Texas, erroneous ideas existed among growers that any variety of oats was good for pasture, no rust resistance was needed, and no seed treatment or fertilizer practices were necessary for good oat pastures. Improvements in cropping practices were started during the early 1950's along with the introduction of 'Victorgrain,' 'Alamo,' 'Mustang,' and later 'Suregrain' and 'Moregrain' oat varieties. A foot-rotting disease incited by *Helminthosporium victoriae* Meehan & Murphy was prevalent from 1954 to 1957 and caused losses in forage yields in Alamo, Mustang, and Victorgrain. Crown rust also attacked these varieties and caused further losses in forage yields.

METHODS

Experiments were designed to evaluate forage yields of small grain varieties under field conditions at Beeville, Texas in 1958. Data included in this paper include seven oat varieties studied from 1958 to 1961. Plots were seeded in October or November and clippings made two or three times during the growing season. Varieties were randomized and replicated four times. Yields on a per acre basis were calculated each year and expressed in pounds of air-dry forage produced per acre. The incidences of rusts and other diseases were recorded each year.

RESULTS

Crown rust, *Puccinia coronata* Cda., was the most destructive rust of oats during the 1958-61 study (Table 1). Stem rust, *P. graminis* Pers. f. sp. *avenae* Eriks. & E. Henn., was not a major factor in this study. The predominant races of crown rust were those in the 213-216 complex. These races attacked Alamo, Mustang, and Mid-South (Table 1) and resulted in severe forage losses during the test period (Tables 2 and 4).

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[1] Cooperative Investigations, Texas Agricultural and Mechanical College and the Crops Research Division, Agricultural Research Service, United States Department of Agriculture. Technical Article No. TA4152, Texas Agricultural Experiment Station.

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Table 1. Response of seven oat varieties to crown rust[a] at Beeville, Texas, 1958-61.

Variety	1958-59	1959-60	1960-61
Suregrain	0	0	0
Moregrain	0	0	30MS[b]
Texas Sel. 245-53-188[c]	0	0	70S
New Nortex	50MR	50MR	50MR
Alamo	70S	70S	70S
Mustang	80S	80S	80S
Mid-South	80S	80S	80S

[a] Notes on crown rust response taken during April each year.

[b] MR = moderately resistant, MS = moderately susceptible, S = susceptible.

[c] (New Nortex x Bond-Rainbow-Hajira-Joanette) x Landhafer.

Table 2. Pounds of air-dry forage produced per acre by seven oat varieties at Beeville, Texas, 1958-61.

Variety	1958-59	1959-60	1960-61	Total
Suregrain	5,000	1,620	3,470	10,090
Moregrain	5,700	1,760	2,554	10,014
Texas Sel. 245-53-188	4,880	1,930	2,955	9,765
New Nortex	3,350	1,520	1,603	6,473
Alamo	2,140	1,310	2,131	5,581
Mustang	2,390	1,020	1,260	4,670
Mid-South	1,940	1,330	1,386	4,656

Table 3. Loss in pounds of forage per acre as a result of a new race of crown rust becoming established on a new oat variety at Beeville, Texas in 1961.

Crop	Suregrain		Moregrain	
year	rust	pounds	rust	pounds
1958-59	0	5000	0	5700
1959-60	0	1620	0	1760
1960-61	0	3470	30MS[a]	2554

[a] 30% of the leaf area was covered by rust with a moderately susceptible pustule.

Table 4. Crown rust reaction and average number of pounds of air-dry forage produced per acre by seven oat varieties at Beeville, Texas, 1958-61.

Variety	Rust[a]	Pounds/acre
Suregrain	0	3363
Moregrain	30MS[b]	3338
Texas Sel. 245-53-188	70S[b]	2955
New Nortex	50MR	2164
Alamo	70S	1860
Mustang	80S	1567
Mid-South	80S	1552

[a] Percent of leaf area covered by rust at last forage harvest.

[b] Rusted only 1 year out of 3.

New races of crown rust appeared during the test period and the resistance of two entries broke down. The 290-295 complex of races attacked Texas Sel. 245-53-188[3] and Moregrain and reduced forage production. Moregrain and Suregrain are similar genetic varieties developed by the Coker Seed Company[4]. During the first 2 years of the test period, these two varieties were equally resistant to crown rust. However, Moregrain became susceptible in 1961 (Tables 1 and 3). Before this time, this variety had yielded more forage than Suregrain, 700 pounds more during the 1958-59 season and 140 pounds more in the 1959-60 season (Tables 2 and 3). During the 1960-61 season, after Moregrain became infected, Suregrain outyielded Moregrain by 916 pounds. The major part of this increase showed in the last clipping when the rust was heaviest. The 30MS infection (Tables 1 and 3) showed up in commercial fields late in the season and many farmers were not aware of the presence of rust in their fields. The forage yields of Texas Selection 245-53-188 were likewise reduced during this same growing season (Tables 1 and 2).

DISCUSSION

Many factors that affect the yield of oat forage were reported previously (4) and were observed in this study. Rainfall, weather conditions, and variety growth habit were predominant factors in forage yields (Table 2). Weather conditions were most favorable for forage production in 1958-59 and least favorable in 1959-60. An intermediate season prevailed in 1960-61. Without irrigation, no control can be placed over these factors. Many diseases can be controlled to increase yields.

The rust disease was established in southern Texas oat fields by urediospores blowing from grain fields in northern Texas, Oklahoma, and Kansas, where the fungus overwinters and may cause damage during the entire winter grazing season. The heaviest rust buildup and loss of forage occur in the late winter and early spring. In some years, the grazing season is cut short by 1 month to 6 weeks. The disease spreads northward from infected fields in the spring. Varieties of grain grown in Mexico, United States, and Canada determine which races of each rust will be predominant.

Why would a moderately susceptible (30MS) type infection reduce the forage yield of 'Moregrain' oats as much as shown in the 1960-61 season? Fletcher, et al. (1) showed that an average infection of wheat stem rust produced 0.5 mg of spores per tiller in a 10-day to 2-week period. Rust infections in southern Texas last from 1 to 4 months. When a plant becomes infected with rust, there is a loss in vigor; but more important recent data (3, 5) have also shown that when a wheat plant becomes infected with stem rust, there is a shift of energy activity from carbohydrate to fat enzyme systems. Rust spores are high in fat. Two and one-half times as much energy is required to produce a gram of fat as a gram of carbohydrate. Much of the energy and raw materials that normally go into building the plant are shunted into fat or rust spores and blown away by winds.

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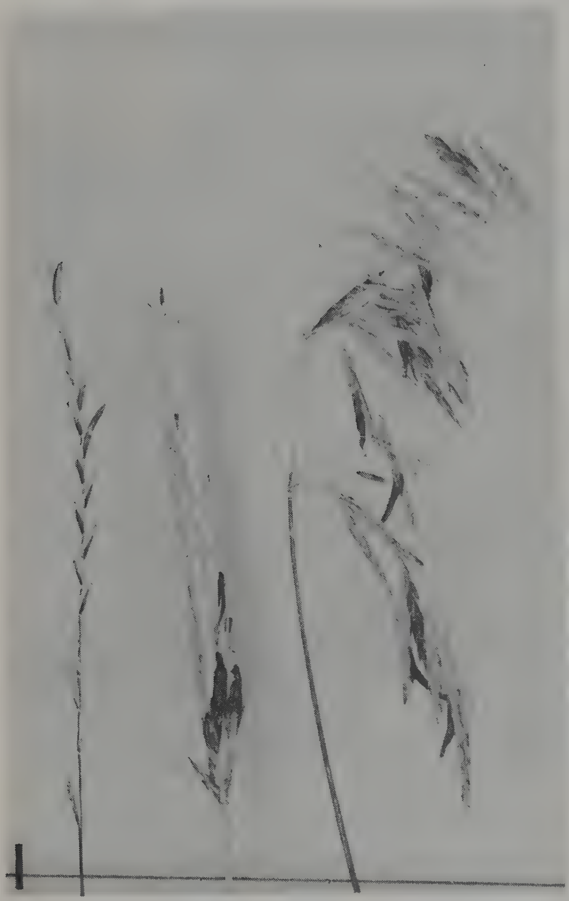
[3] (New Nortex x Bond-Rainbow-Hajira-Joanette) x Landhafer.

[4] Mention of a trade product, equipment, or a commercial company in this publication does not imply its endorsement by the United States Department of Agriculture over similar products or companies not named.

THE OCCURRENCE OF ERGOT ON SMOOTH BROMEGRASS IN MICHIGAN

Delbert L. Bierlein[1]

The appearance of ergot, *Claviceps purpurea* (Fr.) Tul., on smooth bromegrass, *Bromus inermis*, was discovered this year in Eaton County, Michigan near the city of Charlotte. It was observed in a thick stand of grass growing along the roadside and specimens were collected



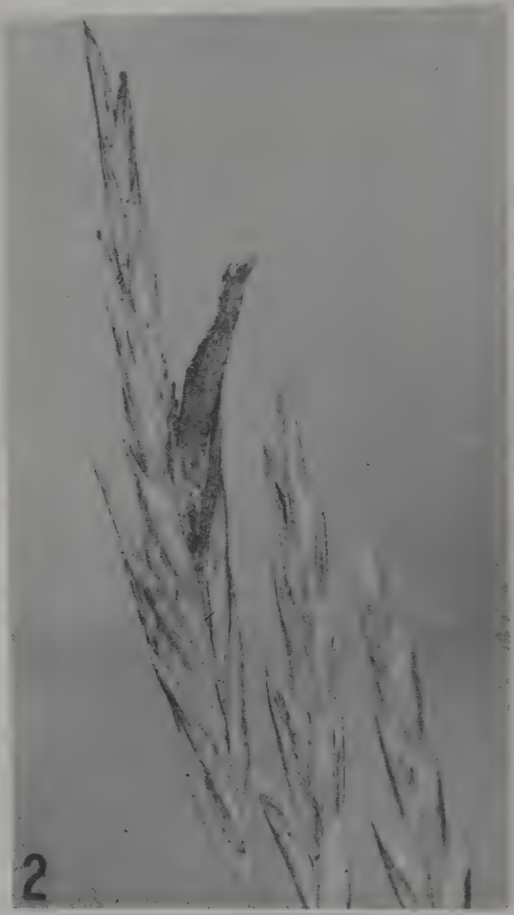


FIGURE 1. From left to right: Ergot on heads of quackgrass, rye, and bromegrass.
FIGURE 2. Close-up showing ergot on bromegrass.

Identification was verified by Dr. H. Spencer Potter, Extension Specialist in Plant Pathology, Michigan State University. Photographs were also taken of the diseased spikes with comparative specimens of rye and quackgrass. The quackgrass specimen was growing adjacent to the bromegrass. The diseased spikes are shown in Figures 1 and 2.

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THE ROLE OF PRATYLENCHUS PENETRANS IN THE DEVELOPMENT OF STRAWBERRY BLACK ROOT ROT[1, 2]

Tseh-An Chen and Avery E. Rich[3]

Abstract

Surveys showed that Pratylenchus penetrans is the most common nematode attacking strawberries in New Hampshire. Populations were lowest in early spring and reached a peak in late summer. Nematodes reduced root growth of strawberries in the greenhouse. They attacked newly initiated roots most readily and caused a severe reduction in growth, but root rot ratings were not changed. Test tube experiments showed that P. penetrans caused the roots of strawberry seedlings to turn black in the absence of other organisms. Under natural conditions nematodes appear to cause primary root injury, then fungi invade the injured tissues. Nematodes tend to leave the injured tissue after it is invaded by fungi, which accounts for low nematode counts in severely injured roots. Thus, although nematodes alone can cause black root rot, fungi also often play a role as secondary invaders.

INTRODUCTION AND LITERATURE REVIEW

During the last thirty years as the investigations of strawberry black root rot disease progressed, evidence has accumulated to show that plant parasitic nematodes may play a role in this complex problem. In 1931 Steiner (10) first reported that meadow nematodes were found in strawberry roots in Florida and Massachusetts. Hildebrand (5) in 1934 noted that nematodes were frequently and consistently associated with diseased strawberry roots and suggested that nematodes might be the cause of strawberry root rot. Recently Braun (1) conducted a survey of plant parasitic nematodes associated with strawberry roots and reported that Pratylenchus spp. were the most prevalent forms in the United States. In the Netherlands, Klinkenberg (6) concluded, after using P. penetrans to inoculate strawberry plants, that nematodes are the primary cause of black root rot because she believed that nematodes must be present before soil fungi are able to attack strawberry roots. Goheen and Smith (4) also showed that strawberry roots taken from nematode-infested soil were badly rotted and showed typical black root symptoms, while those from nematode-free soil were almost free from rot. In contrast, Raski (9) produced evidence to show that P. penetrans was probably not the most important factor limiting the growth of strawberries affected with black root. Miller (8), in Connecticut, stated that the damage to strawberry roots by fungi or nematodes might be independent of the presence of the other.

Preliminary surveys of eight commercial strawberry cultivars and screening of soil collected from root rhizospheres from various locations in New Hampshire revealed a probable relationship between P. penetrans, soil fungi, and the stunted condition of the plants[4]. This paper reports data that cover only the phase involving the action of P. penetrans in the strawberry black root rot complex.

MATERIALS AND METHODS

Two strawberry plantations were selected for nematode population investigations. Eight samples were collected from the Londonderry field and 17 samples were collected from the Durham field in 1959 and 1960. Forty plants from each field were dug randomly on each collection date. Roots were washed to remove soil and debris. The composite root samples were chopped with scissors into small pieces (1/3 to 1/2 cm long) and mixed together thoroughly.

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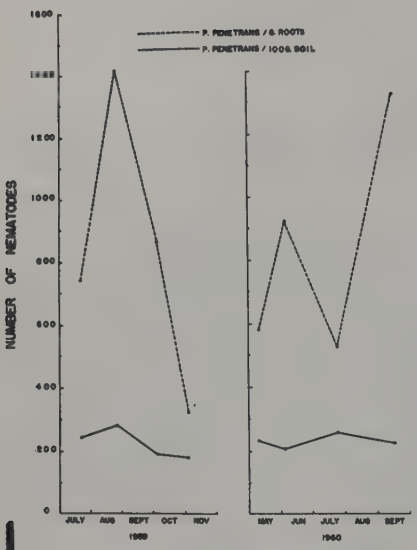
[2] Based on a portion of a dissertation submitted by the senior author to the Graduate School of the University of New Hampshire in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

[3] Formerly Research Analyst, and Plant Pathologist, respectively, New Hampshire Agricultural Experiment Station.

[4] Chen, Tseh-An. 1962. Unpublished data.



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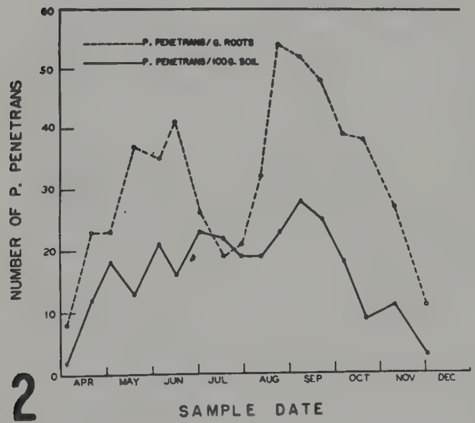

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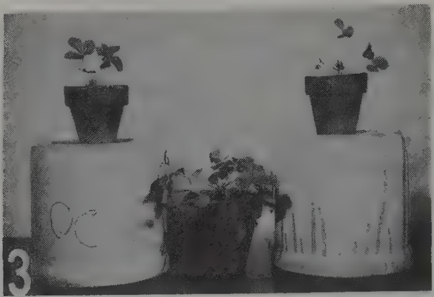

FIGURES 1 and 2. Seasonal fluctuation of nematode populations in strawberry roots and soil in plots at Londonderry and Durham, respectively.

FIGURE 3. Rooting nematode-free runner plants in steam-sterilized soil.

Eight 4-gram representative samples were each placed in a modified Baermann funnel (125 mm) on a single layer of Scott facial tissue paper supported by a copper wire screen. After 24 hours at room temperature, a 50-ml sample from each funnel was drained into a beaker. The funnels were refilled with distilled water and the same process was repeated 24 hours later. Three 10-ml aliquots from each beaker were examined and the nematodes were counted using a counting plate. An average of these counts was recorded and the number of nematodes present in each gram of roots at a given date could be calculated.

The nematodes were extracted from 100-gram soil samples adjacent to the roots by means of a sieve and Baermann funnel technique described by Christie and Perry (3). Nematode counts were recorded as described for the root samples.

Parasitism and pathogenicity of P. penetrans on strawberry roots were studied in the greenhouse as well as in the laboratory. Two tests were conducted in the greenhouse in 1960 and 1961. In the first experiment, virus-free strawberries (cultivar Premier) were collected from university plots and transplanted to 4-inch pots in the greenhouse. Fertilizer (16-32-16) was applied to these plants every 2 weeks and illumination was given to provide at least a 17-hour photoperiod. All plants produced many runners which were "caught" in sterilized pots filled with steam-sterilized, sandy-loam soil (Fig. 3). Two weeks after rooting, plants of uniform size were selected and were severed from mother plants. One 4-inch pot containing one plant was set in another 4-inch pot containing approximately 1 inch of steam-sterilized sand. Nematode inoculum was obtained from infested garden pea (Pisum sativum) roots maintained in the greenhouse. The pea roots were harvested, thoroughly rinsed with running water, mixed and weighed. A few samples were taken to determine the number of P. penetrans present in each gram of roots. The infested pea roots were added to the pots where newly rooted Premier strawberry runner plants were growing. Three 10-plant treatments were set up with the addition of 2000, 1000 and no nematodes to each plant in each treatment. All treated plants were arranged randomly on a greenhouse bench.

In the second greenhouse experiment, nematodes for inoculation were obtained from a pure culture of P. penetrans reared on undifferentiated clover root callus, growing in vitro, on a modified culture medium described by Krusberg (7). Strawberry plants used in the experiment were 6-week-old seedlings (Sparkle-open pollinated). They were grown in sterilized soil in 2-inch pots, watered daily with distilled water and occasionally fertilized with 16-32-16 sterilized soluble fertilizer. Vigorous plants of uniform size were selected. They were removed from the 2-inch pots and the roots were washed in distilled water and checked for uniformity before transplanting into 4-inch pots.

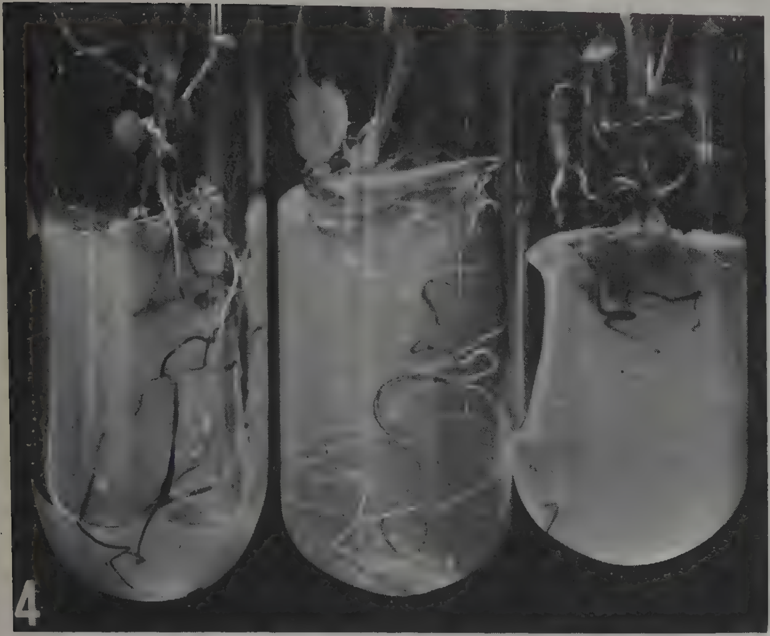
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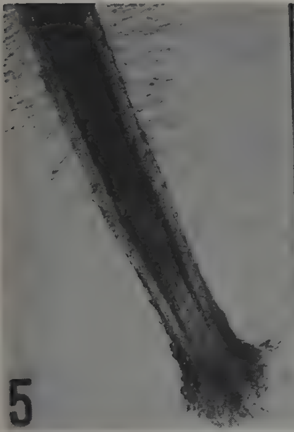
FIGURE 4. Close-up of roots in agar medium. Roots of uninoculated plant (center) are healthy. Roots of nematode-inoculated plants on left and right are black.

FIGURE 5. Healthy root tip of strawberry seedling growing in nutrient agar -- magnified to show root hairs.

FIGURE 6. Root tip of strawberry seedling 24 hours after inoculation with Pratylenchus penetrans at base of plant. The nematodes have clustered at the root tip.

FIGURE 7. Close-up of nematodes feeding on root hairs and epidermis. Root hairs are abnormal.

FIGURE 8. Advanced stage of blackened root tip showing absence of root hairs and partial root disintegration.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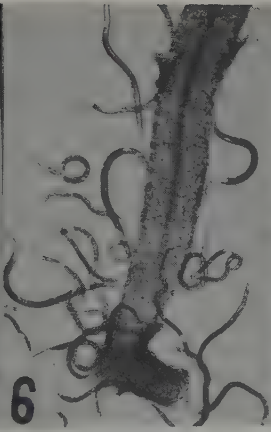
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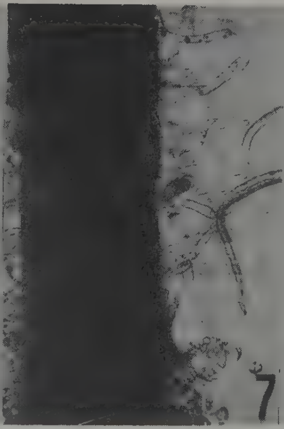
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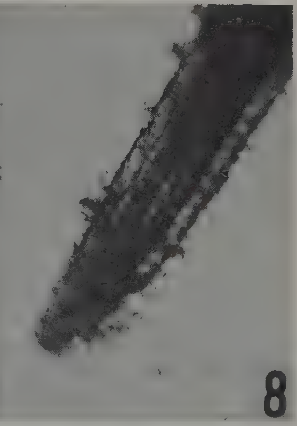
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Plants were divided into two groups: one with normal root system and the other with roots pruned to about 1 1/2 inches. Each group was subdivided into three treatments, that is, with the addition of 2000, 500 and no nematodes per pot. All treatments were replicated 10 times.

Resulting data of both greenhouse experiments were recorded 6 weeks after inoculation.

A test tube technique described by Chen, et al. (2) was used for laboratory experiments. Strawberry seeds were stored in glass vials at 4° C. They were scarified for 15 minutes in concentrated H_2SO_4, rinsed in running tap water for 30 minutes, then surface-sterilized with 10% Clorox (sodium hypochlorite) solution for 5 minutes and rinsed once again with sterilized distilled water. They were then transferred into sterile Petri dishes on moistened filter papers and allowed to germinate at room temperature. Ten to 14 days later, the tiny seedlings were aseptically transferred to test tubes (150 x 18 mm) half filled with sterile nutrient agar medium. When strawberry seedlings had become established in the tubes, usually with four true leaves fully expanded and with root systems well developed, a pure culture of P. penetrans (ranging from 50 to 100 nematodes) was introduced aseptically into the tubes of seedlings. Seedlings in tubes without nematode inoculation served as controls. Macroscopic and microscopic observations were made daily. The progress of parasitism and pathogenicity of P. penetrans on strawberry roots was micro-photographed directly through the tubes without disturbing either the parasites or the hosts.

Table 1. Effect of P. penetrans on the average fresh root weight and root rot rating of strawberry plants grown in the greenhouse for 6 weeks.

Nematodes added	Av. fresh root weight (grams)[a]	Av. root rot rating[a]	No. nematodes per gram root	No. fungi isolated (20 root sections)
2000	4.05	0.8[b]	224	18
1000	4.51	0.7	89	22
0	5.52	0.7	0	14
LSD 5%	0.76			
LSD 1%	1.00			

[a] Each figure represents an average of 10 plants.
[b] 0 = no discoloration; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%.

Table 2. Effect of P. penetrans on the average fresh root weight and black root rot rating of root-pruned and unpruned strawberry plants grown in the greenhouse for 6 weeks.

Treatment and nematodes added	Av. fresh root weight (grams)[a]	Av. root rot rating[a]	No. nematodes per gram root	No. fungi isolated (20 root sections)
Unpruned roots				
2000 nema	5.12	0.7[b]	90	15
500 nema	5.36	0.4	71	18
No nema	5.60	0.4	0	8
Pruned roots				
2000 nema	2.80	0.5	195	19
500 nema	3.45	0.8	91	21
No nema	4.23	0.5	0	11
LSD 5%	0.54			
LSD 1%	0.72			

[a] Each figure represents an average of 10 plants.
[b] 0 = no discoloration; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%.

EXPERIMENTAL RESULTS

Population Levels: Figures 1 and 2 illustrate the seasonal fluctuation of P. penetrans in and about the strawberry roots from the two fields investigated. Nematode populations reached a peak in late August and early September and decreased in October and November. Nematode recovery from strawberry roots was the lowest in the early spring, then it gradually increased during the spring and early summer. Shortly after strawberry fruiting, the nematode population dropped sharply but built up again rather quickly in August. Nematode populations in soil adjacent to strawberry roots fluctuated less throughout the growing season than they did in the roots.

Pathogenicity Study: When pea roots infested with nematodes were used as inocula, no differences in the average root rot rating could be detected between treatments at the end of 6 weeks in the greenhouse. As given in Table 1, there was also little or no difference in fresh root weights between the two treatments in which 2000 and 1000 nematodes were added. However, a highly significant difference occurred in fresh root weight between the check plants and the nematode-infested plants. Plating the strawberry roots from this test yielded a number of fungi. Therefore, it was impossible to be certain that the black root symptoms were due to nematode action alone.

The results from the second greenhouse experiment showed that P. penetrans would attack newly initiated strawberry roots more readily than old roots and they would also inhibit root growth more severely. Table 2 shows that after roots were mechanically damaged (trimmed short) the average fresh root weight of the plants in which 500 nematodes had been added was significantly greater than those where 2000 nematodes were added. The absence of P. penetrans (check plants) resulted in a highly significant increase in root growth over both groups of nematode-treated plants. The addition of 500 and 2000 P. penetrans to the relatively older roots (unpruned) did not appear to influence their growth. Here again, root rot ratings among the treatments showed no difference.

The test tube technique for testing pathogenicity of P. penetrans to strawberry roots was designed to eliminate the confusion occurring in the greenhouse experiments because of fungal contamination. After inoculation, nematodes can be observed moving through the agar medium in test tubes. They clustered near the root tips (Fig. 6) and fed upon the epidermis as well as the root hairs (Fig. 7). Twenty-four hours after inoculation some of the nematodes were entering the roots. Small lesions behind the root cap may be detected at the end of 96 hours by using a dissecting microscope. However, macroscopic lesions were not clearly visible until 14 days later. Typical black necrotic symptoms gradually developed (Fig. 4). Root hairs collapsed and eventually disappeared (Fig. 8). By the end of 2 to 2 1/2 months the black discoloration and necrosis extended the entire length of some of the roots. The plants attempted to produce new roots from the crown but the nearby nematodes were attracted to the new roots and attacked them. As a result, the aboveground parts of the plants became stunted.

DISCUSSION AND CONCLUSIONS

The isolation of large numbers of P. penetrans from blackened roots of strawberry plants and from their rhizospheric soils indicated that this nematode may be involved in a disease complex. Since strawberry is a perennial plant, the two peaks of root production in this area are in early spring and following harvest. Any potential parasites in the rhizosphere could initiate a successful primary invasion of roots at these periods. In this study, patches of rot lesions were often observed on the new roots shortly after their formation. As a result the parasites or pathogens must destroy a large percentage of new roots and feeder rootlets. Secondary invaders may then effect displacement of the primary parasites from the original invaded sites. Repeated examination of strawberry plants when collecting root samples throughout the years and the nematode population counts all indicated that this was actually the case. The formation of new roots in fall and early spring probably accounts for the rise in population while the formation of corky roots in midsummer is unfavorable for population buildup.

In this study, as fungi grew close to the nematode-infested roots, most of the nematodes usually moved away. Fungi usually infected roots more readily at the necrotic portions previously injured by P. penetrans than they did healthy tissues[4]. Thus nematodes can provide necrotic tissue for such fungi which inhabit soil around roots as facultative parasites. It is unlikely, therefore, that P. penetrans would be solely responsible for the incidence of the black root rot. The synergistic effect on the development of this disease complex probably exists only at the initial phase of the infection. However, both parasites must play complementary roles which result in weakened plants.

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DETERMINATION OF DINITRO-ORTHO-CRESOL IN APPLE

K. G. Parker, F. McNicholas, C. A. Bache, G. I. Keenan, and D. J. Lisk

Abstract

Evidence of apple leaf injury from tree applications of dinitro-ortho-cresol led to an investigation of possible fruit penetration by the fungicide. An extraction procedure was devised which effectively removed penetrated residues of the fungicide. June or July application of the chemical at the rate of 0.04 pounds per tree and analyses for surface and internal residues were made. Penetration did occur and residues were barely detectable up to 2 months after application. Neither surface nor internal residues were detectable in the fruit at harvest.

In 1959 and in 1960 apple trees were sprayed with Dow Chemical Company's DN-dry mix No. 2, containing 40% dinitro-ortho-cresol which is effective for eradication of apple scab lesions. Two mixtures were used, 1) containing DN-dry mix No. 2 at 1/2 lb and hydrated spray lime at 3 lb in 100 gallons of water, and 2) containing these materials at the stated concentrations plus the spreader Triton B-1956 at 2 oz in 100 gallons.

Samples of fruit were collected immediately after spraying and at different intervals for analysis. Each sample consisted of one or more pounds of fruit from one or from two trees in different experiments. Apples to be analyzed for surface residues were stored under refrigeration so they would remain firm for surface stripping. Those to be analyzed for internal residues as well as external were frozen until used. This treatment facilitated blending of the fruit during extraction.

Experiment 1: The spray (mixture No. 1) was applied on July 13, 1959 to mature Golden Delicious apple trees in Wayne County in western New York. Samples for analysis were collected on the day of spraying, immediately after the spray application was made, on August 19, and on September 16, 1959. Individual samples were taken from eight sprayed trees and from an equal number of unsprayed ones at each sampling date and analyzed for surface residues of the fungicide.

Westwood and Batjer (2) applied increasing concentrations of the dinitro-ortho-cresol to apple trees and observed that the extent of leaf injury was proportional to concentration. They concluded that leaf injury was a good measure of absorption. If, indeed, absorption of the chemical did occur, penetration of it into the fruit was also possible. To investigate this another experiment (No. 2) was conducted.

Experiment 2: Sprays were applied to mature Rhode Island Greening apples at the same location as experiment 1 the previous year. Applications were made June 22, 1960 and both mixtures No. 1 and No. 2 were used.

Samples were collected immediately after spraying, on July 21, August 23, and September 14, 1960. Four samples were collected at each date from each treatment, and from comparable unsprayed trees, and analyzed for total residue internal and external. Two samples were collected from each treatment for surface residue analysis.

Experiment 3: Sprays similar to those used in experiment 2 were applied to apple trees in the Champlain Valley in northern New York. One application was made on June 14, 1960. Samples were collected immediately after spraying and on June 14, July 12, and August 18. As in experiment 2, samples were collected and suitably treated by the two methods of analysis.

RESIDUE ANALYTICAL METHODS AND RESULTS

A method (1) was available to extract and determine surface residues of the fungicide on apple. An extraction procedure was devised which effectively removed the chemical from apple tissue on a total basis.

The extraction and analysis procedure for determining residues of dinitro-ortho-cresol on a total basis was as follows:

Freeze apples slowly and then thaw them thoroughly. Blend 100 grams of apple with 170 ml of water and then with 200 ml Skellysolve B and 75 ml of isopropanol. Add 170 ml of water and 15 ml of orthophosphoric acid to the mixture and blend until well mixed. Centrifuge the slurry to break the emulsion (15 minutes at 2000 rpm). Aspirate off the hexane layer and filter 100 ml of it into a 250-ml separatory funnel. Extract 100 ml of the hexane with 25 ml of 0.1% sodium bicarbonate. Avoid shaking to prevent emulsions, rather rock the funnel very gently. Repeat the extraction once with 15 ml of the solution and finally with 10 ml. Combine the layers and filter into a 50-ml volumetric flask. Make to volume with 0.1% sodium bicarbonate and mix. Determine the absorbance of the solution at 375 mμ in a 5-cm cell.

For extraction of apples for surface residues of dinitro-ortho-cresol, tumble whole apples for 2 hours with 2 ml of Skellysolve B per gram of apple. Filter the solution and proceed as above beginning with "Extract 100 ml of the hexane solution with sodium bicarbonate . . . ."

The extraction procedures effectively removed the fungicide from apple tissue. Recovery of 0.07 to 0.25 ppm of dinitro-ortho-cresol from apple in 25 determinations averaged 95%.

In experiment 1, surface residues on the day (July 13, 1959) of spray ranged from 0.12 to 0.60 ppm of the fungicide. On August 19 (about 1 month later) the residues ranged from zero to 0.04 ppm. Residues were not detectable at harvest (September 16). In experiment 2 total residues on the day of spray (June 22, 1960) ranged from 1.29 to 1.96 ppm. Surface residues ranged from 0.85 to 1.65 ppm. On July 21, total residues ranged from 0 to 0.08 ppm and surface residues ranged from 0 to 0.04 ppm. On August 23, total and surface residues were no longer detectable.

In experiment 3, total residues on the day of spray (June 14) ranged from 1.80 to 2.75 ppm when spreader was used and from 1.46 to 1.81 ppm on those without spreader. Surface residues were 2.3 ppm. On July 12, total residues ranged from 0.19 to 0.31 ppm when spreader was used and from 0.20 to 0.27 ppm on those without spreader. Surface residues were not detectable from this time on. Residues on a total basis were not detectable on August 18.

In addition to the three experiments described, samples were collected by arrangement with Ivor Kepner of the Barker Chemical Co. from trees sprayed in Niagara County, in western New York. These samples were collected September 26, 1960. One sample was from trees sprayed once, June 11, and two were from trees sprayed on June 11 and on June 22. The spray mixture was similar to those described above without the spreader.

As in the experiments described, no residue was found in these samples. In this case two applications were made as well as one. External analysis of 46 samples of check apples showed an average value of 0.017 ppm. Internal analysis of 27 samples of check apples gave an average check value of 0.163 ppm.

These results amply show that the 40% dinitro-ortho-cresol wettable powder may be applied to apple trees in the summer without detectable residues remaining at harvest. Penetration of the fungicide does occur as evidenced by the higher residues found when the fruit was blended to extract total residues rather than just surface residues.

Acknowledgment: The authors thank N. S. Luepschen and K. D. Hickey for assisting in the field studies.

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EFFECTS OF LIGHT, TEMPERATURE, AND FERTILIZER ON INFECTION OF WHEAT LEAVES BY SEPTORIA TRITICI[1]

Hurley Fellows[2]

The amount of infection by Septoria tritici Rob. ex Desm., the organism causing speckled leaf blotch of wheat, often is very low when the conidia are applied to the wheat leaves in the greenhouse, especially in midwinter. This paper summarizes various experiments conducted in an attempt to increase infection.

METHODS

Conidia of Septoria tritici were produced in abundance on the surface of standard potato-dextrose agar. Differences in the concentration of the ingredients of the potato dextrose did not alter the infection percentages, nor did the addition of dextrose, wheat leaf juice, orange juice, uncooked potato juice, soil solution, six common vitamins, or the medium and mycelium upon which the spores grew. Inoculum was prepared by scraping the spores from the agar surface and mixing them with water. Potted wheat plants were placed in a moist chamber, and the spore suspension was sprayed on the surface of the leaves. A microscopic examination of the water droplets on the leaves after inoculation showed a heavy spore load. A 72-hour incubation period was the shortest in which maximum infection would occur. Notes were recorded after 21 to 30 days when lesions and fruiting bodies were first evident. Inoculated leaves infected and lesions on each leaf were counted. New leaves formed after inoculations were excluded from the records. Two susceptible winter wheat varieties, Westar and Fulhard, and a local isolate of the organism were used.

RESULTS

Conidia 4, 21, and 215 days old when applied to leaves gave similar infections, and young and old leaves and plants were equally susceptible. Evacuation of the inoculated plants three times for 1 minute at 17 inches of mercury to secure closer contact between the leaf surface and the spores increased infection only slightly.

Inoculation with suspensions of pycnospores from lesions on living diseased leaves gave infection percentages practically equal to those obtained from conidia.

One set of plants were kept in the dark during the incubation period of 72 hours by surrounding the moist chamber with black paper; another set was exposed to daylight without direct exposure to the sun, with the light augmented somewhat by white paper reflectors. The data in Table 1 indicate that infection is greatly reduced by darkness.

In another experiment, inoculated plants were exposed to six different daylight intensities of 0, 280, 340, 1040, 1200, and 1300 foot candles during the incubation period. These intensities were provided by placing different numbers of cheesecloth layers over the moist chambers, except the dark chamber, which was covered with black paper. The data in Table 2 indicate that a low light intensity is not favorable to infection.

In still another experiment, the daylight was supplemented by 6 hours of artificial light. In one test, four 40-inch, 40-watt daylight fluorescent tubes were hung over a series of inoculated plants. Four 150-watt reflector Mazda[3] flood lamps were suspended over another set of plants of the susceptible Westar variety. Evidently supplemental artificial light (Table 3) does not increase infection. Flood lights diminished infection considerably, probably as a result of increased desiccation by the additional heat.

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[3] Mention of a trade product, equipment, or a commercial company in this publication does not imply its endorsement by the United States Department of Agriculture over similar products or companies not named.

Table 1. Effect of darkness during incubation on infection of wheat by Septoria tritici.

Wheat variety	Light	No. inoculated	Leaves		Lesions	
			no. infected	% infected	total	per leaf
Fulhard	Full	140	49	35.0	125	2.44
Westar	Full	131	78	59.5	238	3.11
Fulhard	Dark	122	6	4.9	10	1.40
Westar	Dark	144	11	7.6	12	1.06

Table 2. Effect of light intensity during incubation on the infection of 'Westar' wheat leaves by Septoria tritici.

Observation	Light intensity in foot candles					
	0	280	340	1040	1200	1300
Percent leaves infected	11.0	67.4	64.9	79.5	83.0	88.7
Number of lesions per leaf	1.17	3.05	3.35	4.83	4.83	5.71

Table 3. Effect of supplemental light on the incidence of speckled leaf blotch on artificially inoculated 'Westar' wheat in the greenhouse.

Leaves	Daylight plus		
	nothing	fluorescent light	flood lamp light
Total number	1095	679	962
Av. percent infected	57.3	56.5	24.5
Av. number lesions per infected leaf	2.77	2.70	1.42

EFFECT OF FERTILIZERS

Under field conditions, natural infection apparently was greater where growth was vigorous. In the greenhouse, 4-inch pots of wheat received 1/2 teaspoon each of Vigoro applied to the soil surface 0, 7, 9, 11, or 13 days before inoculation. Each treatment involved six pots each containing four plants. Both plant growth and leaf infection (Table 4) were greatly increased by the fertilizer.

Table 4. Effect of fertilizer on infection of wheat leaves by Septoria tritici.

Period fertilized before inoculation (days)	No. leaves inoculated	% leaves infected	No. lesions per infected leaf
0	153	6.1	0.70
7	169	73.6	3.06
9	214	55.4	2.67
11	222	60.0	2.86
13	214	41.4	2.21

Table 5. Effect of high and low temperature during incubation on the infection of wheat leaves by Septoria tritici.

Experiment no.	Low temperature		High temperature	
	% infected leaves	no. lesions per leaf	% infected leaves	no. lesions per leaf
1	10.3	1.47	9.4	1.52
2	30.1	1.00	28.0	1.00
3	5.3	1.26	3.8	1.04
4	29.2	1.62	69.3	2.29
5	17.9	1.25	62.7	2.51
6	54.7	2.44	67.5	3.16
7	44.7	1.55	69.3	3.58
Average	27.5	1.51	44.2	2.15

EFFECT OF TEMPERATURE

In 3 years, seven tests were conducted to determine the effect of incubation temperature on the infection of wheat leaves by Septoria tritici. The low temperature was obtained by introducing outside cold air into a thermostatically controlled double-walled glass chamber. The leaves of each potted plant were confined in lantern globe moist chambers. The low temperature varied from 40° to 50° F with an average of 44°. The results (Table 5) are compared with those at the higher greenhouse temperature, which averaged 71°. The higher temperature favored infection in four of the seven experiments, but rather heavy infection sometimes occurred at the lower temperature.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, IN COOPERATION WITH THE KANSAS AGRICULTURAL EXPERIMENT STATION, DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, MANHATTAN, KANSAS

CUCUMBER MOSAIC VIRUS ON SAFFLOWER[1]

J. M. Klisiewicz[2]

In May 1962, mosaic symptoms were observed on foliage of safflower, *Carthamus tinctorius*, grown in the Sacramento Valley of California. A survey of the production area revealed that the disease occurred sporadically in commercial fields. Similar symptoms had been observed since 1958 on safflower grown in production areas of California, Arizona, and Utah[3]. A mosaic occurring on leaves and bracts of safflower grown in Israel was found by Nitzany (2) to be caused by cucumber mosaic virus, CMV.

Symptoms on foliage (Fig. 1) consist of a light- and dark-green mosaic on upper leaves and involucral bracts and irregular flecks of light green interspersed with dark green on shaded, lower leaves. Some leaves become blistered and distorted. Affected plants develop to maturity, although slightly stunted in growth.

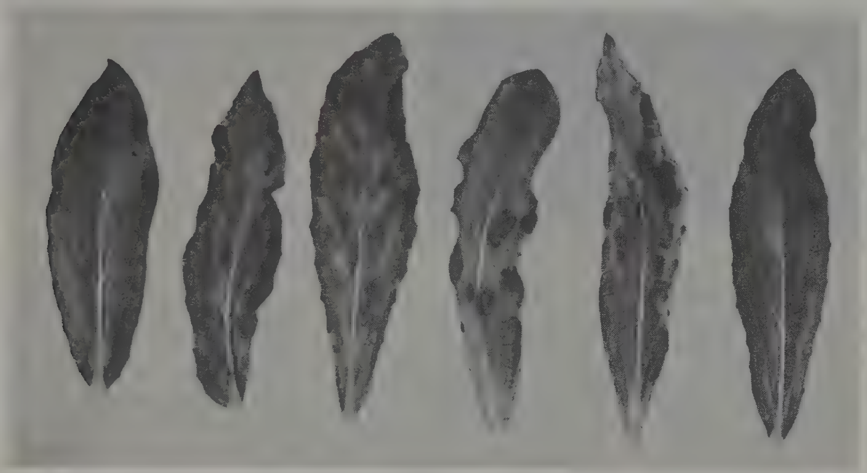

FIGURE 1. Symptoms of cucumber mosaic virus on safflower leaves. Healthy leaves (left and right). Diseased leaves show mosaic pattern, blistering, and distortion (center).

Table 1. Symptoms on plants inoculated with virus isolated from safflower.

Species inoculated	Local lesions	Systemic symptoms
Vigna sinensis	reddish-brown	
Cucumis sativus		yellow mottle, leaf distortion
Chenopodium amaranticolor	pale-yellow	
Zinnia elegans		leaf mottle
Nicotiana glutinosa		severe mottle, leaf distortion, blistering and stringing
Capsicum annuum		leaf mottle
Nicotiana tabacum		mottle, vein clearing
Datura stramonium	pale-green	
Cucurbita pepo		severe mottle of leaves
Carthamus tinctorius		light- and dark-green mosaic, leaf blistering and distortion

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[3] K. E. Mueller. 1959. Annual Report on Safflower Diseases. ARS.

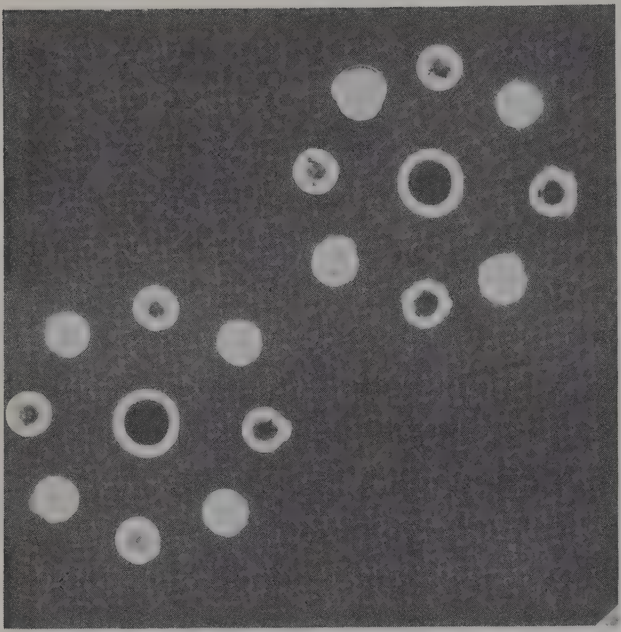

FIGURE 2. Precipitation lines in agar between crude extracts from virus-infected safflower tissue (four small wells in each test), and antiserum for CMV (large center well). Small wells at corners of fused lines contain extract from healthy safflower.

Experimental tests were conducted for identifying the virus in safflower.

In addition to safflower, a number of plant species within the host range of CMV were selected for inoculation with the virus isolated from safflower. Sap was extracted from diseased plants collected in the field by grinding leaves in a mortar to which 2 ml of .01 M phosphate buffer, pH 7.0 was added. Inoculations were made with a cotton pad on leaves and cotyledons sprinkled with corundum powder. Similarity between symptoms (Table 1) observed during a 3-week period after inoculation and symptoms produced by CMV on the same hosts (2, 4) suggested that the mosaic on safflower was likely produced by CMV.

The agar gel-diffusion method (3), whereby antigens can be tested against corresponding antisera, has been used for serological reactions between some plant viruses and corresponding antisera (5). Grogan and Kimble (1) identified CMV in crude extracts from CMV-infected plants by testing the extracts against an antiserum for CMV in agar-diffusion plates. The technique was used to substantiate identity of the virus in safflower. Sap was extracted from virus-infected safflower collected in the field, and tested against antisera for CMV and alfalfa mosaic virus. The formation of a precipitation line between wells containing antiserum and each well containing crude sap indicated presence of CMV (Fig. 2). No lines formed opposite wells containing sap from healthy plants. A positive reaction occurred with crude sap tested from several collections of virus-infected safflower. No reaction was indicated in agar plates in which extracts were tested against antiserum for alfalfa mosaic virus. Results of the test establish identity of the virus causing mosaic on safflower grown in California and the western States as CMV.

Safflower varieties US 10, Gila, Nebraska 10, Pacific 7, Pacific 1, and Pacific 2 are susceptible to the CMV isolate from safflower. Twenty 10-day-old plants of each variety were inoculated by rubbing inoculum on the first true leaves. Systemic symptoms appeared 10 to 14 days after inoculation on approximately 50% of the plants of each variety. The virus was transmitted to safflower by the green peach aphid, Myzus persicae (Sulz.). Aphids were transferred to 10 healthy seedlings of varieties US 10 and Gila, after a feeding period of 10 minutes on a virus-infected safflower plant. Eighty percent of the plants developed typical mosaic symptoms.

Seed was harvested from virus-infected plants in the field and planted in the greenhouse, where several hundred plants were observed to the flowering stage for development of disease symptoms. No diseased plants were produced from the seed to indicate seed transmission.

Widespread occurrence of CMV on safflower would depend largely on: prevalence of wild or other hosts of the virus, heavy aphid populations on CMV-infected hosts, and flights of the infective aphids to safflower early in the growing season.

Materials furnished by Dr. R. G. Grogan and K. A. Kimble, Department of Plant Pathology, University of California, Davis are acknowledged.

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A NEW GREENHOUSE TECHNIQUE FOR EVALUATING FUNGICIDES FOR CONTROL OF COTTON SORE-SHIN[1]

Hany M. Elsaid and J. B. Sinclair[2]

Abstract

A new technique was developed to screen soil fungicides under greenhouse conditions for control of Rhizoctonia solani Kuehn, the cause of sore-shin (damping-off) of cotton seedlings. The technique simulates field conditions by placing infested soil on the edge of treated soil and measuring the progress of disease development on cotton seedlings. This technique proved to be effective in screening 18 soil fungicides in both steam-sterilized and nonsterile soil. These fungicides differed in their behavior, depending on the kind of soil used. The new technique has advantages over other methods by providing a better evaluation of phytotoxicity against pre- and post-emergence damping-off. In these studies, pentachloronitrobenzene (PCNB) gave the most desirable results, followed by tributyltin chloride of abietylamine ethylene oxide (Tin-San).

INTRODUCTION

A limited number of techniques used to evaluate soil fungicides in the greenhouse have been reported (1, 3, 5, 7). A clay pot technique was used to screen soil fungicides for control of root-rotting organisms isolated from infected lily, poinsettia and other ornamentals (5), and also to screen soil fungicides for the control of bean root rot (1). Sinclair (7) used greenhouse flats to screen various soil fungicides for control of sore-shin of cotton seedlings, and Ranney and Bird (3) used a similar technique to study the effect of temperature on the effectiveness of soil fungicides.

This report describes a new greenhouse method for screening soil fungicides used for the control of cotton sore-shin. This work is a portion of a thesis written in partial fulfillment of the requirements for a Master of Science degree (2).

MATERIALS AND METHODS

The preparation of inoculum for soil infestation in this study was essentially the same as that used by Sinclair (6, 7). Sinclair's isolate T (6, 7, 8) of Rhizoctonia solani Kuehn was used for all experiments.

Flats of moist soil were infested by: removing the upper layer of the soil; distributing the fungus suspension evenly over the surface of the soil remaining in the flat; and replacing the displaced soil. These flats were incubated for 2 weeks in the greenhouse and served as a source of infested soil. The soil in the flats was kept moist at all times.

The new method for screening fungicides was developed as follows. Fungicides were mixed thoroughly with flats of soil. After mixing, the soil was leveled and pressed until firm. Then, approximately 1600 cc of treated soil in a band was removed 4.5 cm wide and the length and depth of the flat, and an equal amount of infested soil was used to replace it. Each flat was planted with seven rows containing 50 seeds per row. Each row was considered a replicate. These flats were kept moist throughout the experiment, which lasted 3 to 4 weeks. The total distance in centimeters in which diseased plants occurred was measured for each row several times during each experiment. The data were subjected to statistical analysis. This technique was thought to approximate field conditions where soil had been treated with soil fungicides for control of sore-shin.

The fungicides, with their percent active ingredient, tested were: 50% captan, 70% dimethylaminobenzenediazo sodium sulfonate (Dexon), 50% dichlone, 80% maneb, 75% pentachloronitrobenzene (PCNB), 65% thiram, methylmercury dicyandiamide (EP-126B), 10% N-trichloromethyl mercaptobenzoxazolone and 10% pentachloronitrobenzene (EP-177D), 25% 2, 2'-methylenebis (3, 4, 6-trichlorophenol) (Nabac M1770), 1.25% hexachlorophene and 50% captan (Nabac-captan), 70% trichlorodinitrobenzene (Chemagro 2635), 50% and 75% 3, 3, 4, 4-tetrachlorotetrahydrothiophene-1, 1-dioxide (DAC 649), 100% tributyltin chloride of abietylamine ethylene oxide

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[2] Respectively, graduate student and Associate Professor of Plant Pathology, Louisiana State University, Agricultural Experiment Station, Baton Rouge.

(Tin-San), 5%, 10% and 25% methyl-4-dimethylamino-3, 5-xylyl carbamate (Zectran), 25% S-(2-pyridyl-1-oxide)-S'-trichloromethyl disulfide hydrochloride (Olin 2086), 25% copper derivative of 12, -naphthoquinone-2-oxime (Cyanamid 28720), ammonium salt of dichloroacetone bisulfite (Norwich U-587), and 10% phenylmercuric acetate (General Chemical Tag).

Dust and wettable powders were applied at an equivalent weight of 5 pounds per acre. Liquid fungicides were applied at a rate of 4 quarts per acre.

The fungicide suspensions were prepared by either mixing 0.36 g of dust and wettable powders or 0.61 cc of liquid fungicides in 500 cc of distilled water. Steam-sterilized and nonsterilized field soil were used and compared. Nontreated checks served as controls.

A preliminary test was made to determine which of three possible designs would give desired results, that is, the 4.5-cm band of infested soil could be placed in a greenhouse flat as either: (A) a single row along one side; (B) a single row in the center; or (C) as two rows, one on each side. In this preliminary experiment the three designs were compared using the fungicides PCNB, 2635, EP-126B and Tin-San. Readings were taken at 16, 18 and 20 days after planting. Pattern A of the technique described above appeared to be the best to evaluate soil fungicides (2). The main disadvantage of patterns B and C was that they required two readings for each row, whereas pattern A required only a single reading. Other disadvantages were that pattern C had two sources of inoculum and plants were killed too rapidly for a fair evaluation and pattern B split the noninfested area into two parts and again plants died too rapidly.

A series of three tests using pattern A was made to evaluate the effectiveness in controlling pre- and post-emergence damping-off of the 18 fungicides listed and their phytotoxicity to cotton seedlings.

The first test used steam-sterilized and nonsterile infested soil and compared four fungicides: PCNB, 2635, EP-126B and Tin-San.

In the second experiment the fungicides listed in Tables 1, 2 and 3 were used. Checks consisted of a nontreated-noninfested flat and a nontreated-infested flat. Only steam-sterilized soil was used and readings were taken at 17, 19 and 21 days after planting. Data taken at 17 days are reported.

Steam-sterilized and nonsterilized soil were compared in the third test. The fungicides used are listed in Tables 1, 2 and 3. The same types of checks were used as in the second experiment. Readings were taken at 14, 16 and 20 days after planting. Data taken at 14 days are reported.

Table 1. Pre-emergence damping-off control exhibited by various fungicides in greenhouse flats of steam-sterilized or nonsterile soil infested with R. solani expressed as mean[a] area in cm in which no seedlings emerged.

Treatment and test number					
Sterile				Nonsterile	
Treatment	II	Treatment	III	Treatment	III
Check 1[b]	8.0	DAC-649 50	1.1	Zectran-5	2.1
PCNB	8.9	DAC-649 75	1.7	Dexon	2.3
U-587	9.0	2086	1.9	2635	2.3
DAC-649 50	9.5	Thiram	2.0	EP-177D	3.7
Nabac 5	10.0	Zectran-10	2.1	Zectran-25	4.0
Thiram	10.2	EP-177D	2.1	Zectran-10	4.3
Nabac 25	10.3	Nabac 5	2.4	28720	4.3
28720	10.3	28720	2.7	DAC-649 75	5.3
Dexon	10.4	2635	2.9	Nabac 25	6.0
DAC-649 75	10.7	Dexon	2.9	Thiram	6.3
Zectran-5	12.7	Zectran-25	2.9	U-587	6.7
Zectran-10	13.1	Zectran-5	3.9	2086	7.3
Tag	27.6	U-587	4.4	PCNB	7.1
		Nabac 25	4.7	DAC-649 50	7.1
		Check 1[b]	4.9	Nabac 5	7.0
		PCNB	7.3	Check 1[b]	8.6
Check 2[c]	0.0	Check 2[c]	0.0	Check 2[c]	0.0
LSD .05	0.1		0.7		0.7
LSD .01	0.1		0.9		0.9

[a] Mean of seven replicates.

[b] Nontreated-infested.

[c] Nontreated-noninfested.

Table 2. Mean[a] percentage stand from soil mixed with various fungicides in greenhouse flats of steam-sterilized and nonsterile soil.

Sterile				Nonsterile	
Treatment	II	Treatment	III	Treatment	III
PCNB	65.2	Check 2[c]	82.0	Check 2[c]	75.2
Check 2[c]	64.6	Zectran-25	74.0	2635	73.8
Check 1[b]	62.6	Dexon + PCNB	72.2	Dexon + PCNB	68.0
28720	56.2	2635	72.0	28720	65.2
U-587	54.6	DAC-649 50	69.6	Zectran-5	62.8
Nabac 5-10	54.0	DAC-649 75	67.8	Zectran-10	62.0
DAC-649 75	52.0	Zectran-5	67.4	Zectran-25	60.6
Nabac 25	50.6	EP-177D	67.2	Nabac 5-10	60.2
Dexon + PCNB	49.8	Check 1[b]	66.0	DAC-649 50	56.8
Zectran-10	48.2	Nabac 5-10	65.4	DAC-649 75	56.2
Thiram	46.0	PCNB	64.0	EP-177D	56.0
Zectran-5	45.8	Nabac 25	62.0	Nabac 25	56.0
DAC-649	44.6	Zectran-10	62.0	Thiram	55.8
Tag	15.2	U-587	60.6	U-587	54.8
		Thiram	57.8	PCNB	54.2
		2086	52.2	2086	53.4
		28720	42.8	Check 1[b]	53.4
LSD .05	2.8		1.2		1.2
LSD .01	2.2		1.6		1.6

[a] Mean of seven replicates of 50 seed each.

[b] Nontreated-infested.

[c] Nontreated-noninfested.

Table 3. Post-emergence damping-off control exhibited by various fungicides in greenhouse flats of steam-sterilized and nonsterile soil infested with *R. solani* expressed as mean[a] area in cm in which diseased cotton seedlings occurred.

Sterile				Nonsterile	
Treatment	II	Treatment	III	Treatment	III
PCNB	12.6	PCNB	14.3	2635	16.1
Check 1[b]	16.8	DAC-649 75	22.4	28720	22.1
Dexon + PCNB	16.4	Zectran-25	22.7	Dexon + PCNB	23.7
28720	20.2	Nabac 5-10	23.6	PCNB	24.9
DAC-649 75	21.7	DAC-649 50	23.7	Zectran-5	25.0
Nabac 25	21.9	Thiram	26.1	Nabac 5-10	28.0
Thiram	22.2	U-587	27.3	DAC-649 50	29.8
Nabac 5-10	22.4	Check 1[b]	27.7	Thiram	30.3
U-587	23.4	28720	29.0	Check 1[b]	32.0
Zectran-10	24.6	2635	32.0	DAC-649 75	32.0
Zectran-5	24.7	EP-177D	32.0	EP-177D	32.0
DAC-649 50	25.5	Nabac 25	32.0	Zectran-10	32.0
Tag	32.0	Dexon + PCNB	32.0	Zectran-25	32.0
		Zectran-5	32.0	2086	32.0
		Zectran-10	32.0	U-587	32.0
		2086	32.0		
Check 2[c]	0.0	Check 2[c]	0.0	Check 2[c]	0.0
LSD .05	0.6		1.1		1.1
LSD .01	0.8		1.5		1.5

[a] Mean of seven replicates.

[b] Nontreated-infested.

[c] Nontreated-noninfested.

RESULTS

The data from studies to control pre-emergence damping-off are based on the area measured in centimeters where no germination occurred, in or near the infested soil. In test II, pre-emergence damping-off was greater in treated plots than in the nontreated check. In the third test, all fungicides used except PCNB in sterile soil showed control of pre-emergence damping-off (Table 1). A difference in behavior of the fungicides in sterile and nonsterile soil was noticed. Damping-off was generally more severe in nonsterile soil. All fungicides used in this test showed better control for damping-off in sterile soil except Zectran-5, which showed better control in nonsterile soil and 28720 which showed no difference in control.

The data from stand counts are given in Table 2 as percentage stand from 50 seed. All chemicals tested showed some degree of phytotoxicity when compared with nontreated-noninfested checks, except PCNB in Test II using sterile soil. In Test III, however, PCNB exhibited some phytotoxicity. Stand counts were generally better in steam-sterilized soil than in nonsterile soil. The chemicals showing the least phytotoxicity were 2635, 28720, Dexon + PCNB and Zectran-25 in sterile soil and 2635, 28720 and Dexon + PCNB in nonsterile soil.

Post-emergence damping-off control by various fungicides was studied in three tests. In the first test PCNB, Tin-San, 2635 and EP-126B were compared in sterile and nonsterile soil. In both types of soil at 20 days after planting PCNB gave the best control, followed by Tin-San, 2635 and EP-126B, decreasing in that order. The differences were highly significant between each treatment in both soils except for PCNB and Tin-San in nonsterile soil (2).

The results from post-emergence damping-off studies in the second and third test are summarized in Table 3. The behavior of soil fungicides differed according to whether they were tested in steam-sterilized or nonsterile field soil, and control exhibited by the various fungicides generally fell in four groups: 1) those fungicides that gave better control in steam-sterilized soil, for example, PCNB, Tin-San, and DAC-649 75; 2) those fungicides that gave better control in nonsterile soil, for example, 2635, 28720, Nabac 25, Zectran-5, EP-126B, and Dexon + PCNB, and Dexon; 3) those fungicides that gave control in both soil treatments, for example, PCNB, thiram, Nabac 5-10, and DAC-649 50; 4) those fungicides that gave no control in either soil treatment, for example, 2086, Zectran-10, Tag, U-587 and EP-177D.

DISCUSSION

The technique, developed in this work, had the following advantages:

1. The soil is thoroughly mixed with fungicides, and soil so treated is more homogeneous than it is in the in-the-furrow method (7).
2. This technique gave a better evaluation for pre-emergence damping-off because the effect of the organisms on germination can be measured without the phytotoxic effect of the fungicides.
3. This new technique gives better evaluation for post-emergence damping-off because more than one reading can be taken.
4. The phytotoxicity shown by each test chemical is evaluated better because most of the soil in the flat is treated with the test chemical and only a strip of infested soil is placed at the edge of the flat. Stand counts give a better indication of phytotoxicity than does the in-the-furrow method (7), because in the latter method stand counts are affected by the phytotoxicity of chemical plus pre-emergence damping-off caused by the test organism.
5. The spread of the disease from infested area to noninfested area can be evaluated using this technique.

This new technique was used to screen soil fungicides in sterile and nonsterile soil. Cases where better control was obtained in nonsterile soil may be due to the fact that in sterile soil only the effect of the chemical on _R. solani_ is measured, while in nonsterile soil the control of _R. solani_ may be increased because of the presence of microorganisms antagonistic to _R. solani_. Where one chemical may inhibit these antagonistic organisms, another may not show inhibition. Richardson (4) found that where thiram persisted in sandy soil it had a selective action against other fungi. In the work reported here, a fungicide may be selective against certain fungi in the soil. Therefore, fungicidal control of organisms inciting damping-off, other than _R. solani_, will give better overall control because of the effect of antagonistic microorganisms on _R. solani_.

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AMERICAN DAGGER NEMATODE (XIPHINEMA AMERICANUM) ON COFFEE IN GUATEMALA, WITH SUGGESTIONS FOR NEMATODE CONTROL IN NURSERIES

Gerald Thorne and Eugenio Schieber[1]

This report is based on a limited survey of typical coffee Fincas (farms) located in three coffee areas: Los Brillantes, El Tumbador, and Antigua Sacatepequez.

Previous investigations by Schieber and Sosa (2) and by Chitwood and Berger (1) demonstrated that root-knot nematodes, Meloidogyne spp., and root-lesion nematodes, Pratylenchus spp., are widely distributed through the coffee areas of Guatemala. These nematodes were frequently observed during the present survey and, in addition, the American dagger nematode, Xiphinema americanum, was found in almost every soil sample, often in great numbers. This ectoparasitic species was especially numerous in one Finca of the Los Brillantes area where 280 acres are being abandoned. Both root-knot and root-lesion species also were present in this Finca and the combined infestation produced a complex of root rot symptoms which had practically destroyed all roots.

Fincas of the Tumbador area were generally in good condition, with only occasional small groups of trees exhibiting symptoms of nematode injury. Xiphinema americanum usually was present in this area, but under the cultural practices followed there was little evidence of the severe decline and dieback noted in many orchards in the United States. The presence of this species should not be minimized, however, since in the course of their feeding the nematodes puncture and destroy cells, leaving tiny lesions through which fungi and bacteria may enter later.

Direct control of nematodes in established plantings is impossible because of the difficulty in applying nematocides. Maintenance of high fertility and proper cultural practices are essential and in most of the Fincas visited these practices are obviously successful.

Nurseries received the most attention during the survey because in them severe losses have occurred frequently on several Fincas. Clean, strong young trees are imperative for transplanting if production is to be maintained or increased.

Control in nurseries is feasible because of the small areas involved and their accessibility for application of soil fumigants. Direct application of fumigants to nursery soil works best when a plastic cover is used to retain the fumes and prevent escape of nematodes in the top 2 or 3 inches of soil where lethal concentrations do not accumulate. At least 40 to 50 gallons of 1,3-dichloropropene-1,2-dichloropropane mixture (D-D) or equivalent fumigants per acre should be applied.

Seedlings are frequently grown in individual bags, which is a most satisfactory method of control. All bagging soil should be thoroughly fumigated under a plastic cover for 3 or 4 days, then thoroughly aerated before filling the bags. Trees grown in this manner can be transplanted with a minimum of injury and, with proper fertility, should become well established before nematodes accumulate about them.

One grower is experimenting with 1,2-dibromo-3-chloropropane (Nemagon) granules in the planting hole. This should be given careful attention since a superior root system may be produced by reducing nematode populations during the critical period -- when the young trees are becoming established.

Another grower said that he never plants a young tree where an old one has stood; this should be adopted as a standard practice. The huge colonies of nematodes that accumulate about the roots of old trees readily transfer to the roots of young trees planted in their places.

Growers should be alert for trees in infested areas that exhibit good growth, an indication of possible resistance or immunity; such resistance or immunity has been demonstrated in the tea-producing areas of Ceylon.

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NEMATODES ASSOCIATED WITH WILD YAM, DIOSCOREA SP., WITH SPECIAL REFERENCE TO THE PATHOGENICITY OF MELOIDOGYNE INCOGNITA INCOGNITA[1]

W. R. Jenkins and G. W. Bird[2]

Abstract

Various nematodes were found associated with the wild yam, Dioscorea sp., in Guatemala. Among these were Meloidogyne incognita incognita, Pratylenchus brachyurus, and Criconemoides n. sp.; all were common and occurred frequently in large numbers. Infection of feeder roots by Meloidogyne resulted in typical giant cell formation and normal nematode reproduction. Infection of storage roots resulted in formation of "stone cells," which were comprised of a mature female and egg mass enclosed in a wound periderm cyst-like "cell." Yam infected with root-knot nematodes was stunted, with galling and malformation of storage roots.

INTRODUCTION

There has been considerable interest in recent years in the establishment of the wild yam, Dioscorea spp., as a commercial crop in Guatemala. Storage roots of this plant contain the steroid diosgenin which is easily converted to the cortisone group of drugs. Since these plants are principally tropical, the possibility of nematodes adversely affecting their growth has been of prime consideration.

A number of nematodes have been mentioned as being associated with various species of Dioscorea. These include Meloidogyne javanica, M. arenaria, M. incognita incognita, Rotylenchus bradys, R. blaberus, Pratylenchus pratensis, and Tylenchus sp. (6, 7, 8, 9). No detailed pathogenicity has been reported, although Schieber and Lassmann (9) stated that M. incognita incognita caused "severe symptoms" on both roots and aerial parts of the wild yam and posed a serious threat to the establishment of this crop in Guatemala.

MATERIALS AND METHODS

Survey: Samples of both soil and roots were collected from the Finca Las Delicias, Retalhuleu, Guatemala and the Finca Concepcion Buena Vista, St. Lucia, Cotz., Guatemala. Care was taken to assure that only roots of Dioscorea spp. and soil immediately about them were included. These samples were processed by a modification of the centrifugation-flotation technique developed by Caveness and Jensen (1). Nematodes were identified to genus and, where possible, to species.

Pathogenicity: Seedlings of a wild yam, Dioscorea sp., infected with Meloidogyne incognita incognita were fixed in FAA, dehydrated by a tertiary butyl alcohol series, imbedded in Fisher's Tissuemat, and sectioned on a rotary microtome at 10μ thickness for feeder roots and on a sliding microtome at 20μ thickness for large storage roots. Sections were adhered to slides

FIGURE 1. Seedlings of Dioscorea sp. infected with Meloidogyne incognita incognita (right) and uninfected (left).

FIGURE 2. Photomicrographs of stained sections of Dioscorea sp. a -- Feeder root containing mature females of Meloidogyne incognita incognita, associated giant cells, and egg masses. Note the wound periderm formed by the host in the vicinity of the egg masses. b -- An uninfected feeder root with normal development. c -- Storage root with a "stone cell" which is a periderm-encased female of Meloidogyne incognita incognita and its associated egg mass. d -- Uninfected storage root normally developed.

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers-The State University, Department of Entomology.

[2] The authors are indebted to Miss Ellen Powers for her technical assistance during the course of this investigation.

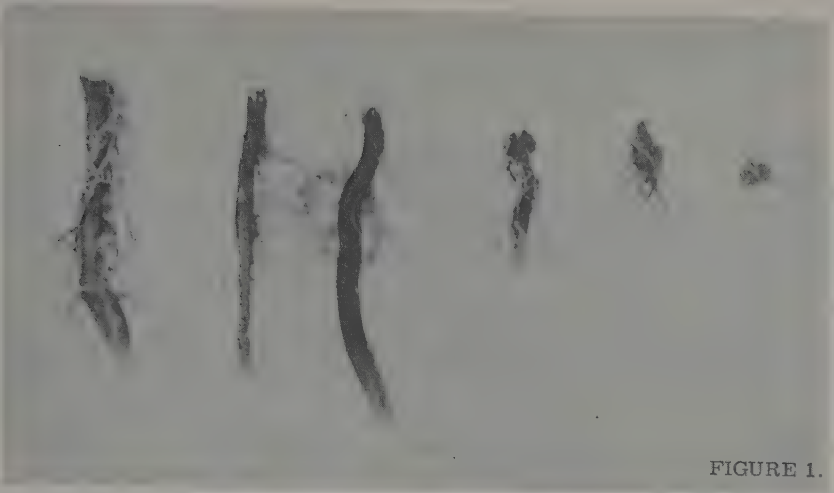

FIGURE 1.

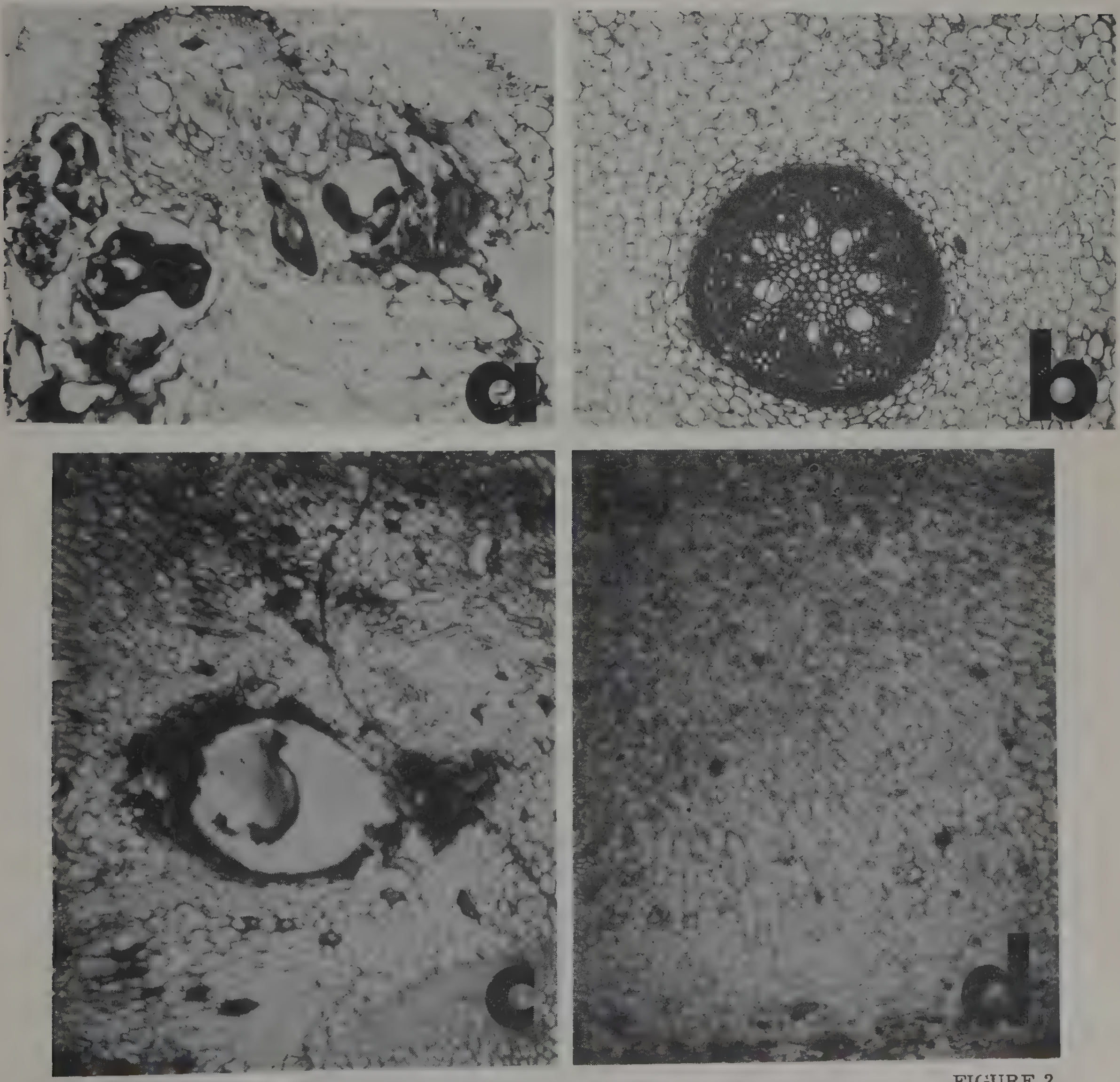

FIGURE 2.

(See legends on opposite page)

with Haupt's adhesive, stained in a safranin-fast green combination with Harris' Hematoxylin, and mounted in Fisher's Permount.

RESULTS AND DISCUSSION

Survey: In 90 samples, the following plant-parasitic nematodes were observed in or closely associated with yam roots: *Pratylenchus brachyurus*, *Criconemoides* n. sp., *Xiphinema americanum*, *Meloidogyne* sp., *M. arenaria*, *M. javanica*, *M. incognita incognita*, *Helicotylenchus* sp., *Trichodorus* sp., and *Rotylenchulus reniformis*. Of these, *Criconemoides* n. sp., *Meloidogyne incognita incognita*, and *Pratylenchus brachyurus* occurred most often and in the largest numbers.

Pathogenicity: Seedlings of yam parasitized by *Meloidogyne* were severely stunted and their roots were distorted and galled (Fig. 1). Both feeder and storage roots were invaded. Histological examination of parasitized feeder roots revealed the presence of mature females, egg masses, and larvae. There was typical giant cell formation, disruption of xylem elements, and cortical destruction similar to that described on other infected crops (2, 3, 4, 5). When egg deposition occurred within roots, a wound periderm was formed by the host in the area of the egg mass (Fig. 2a). However, larvae were able to emerge from host tissue and reinfect other sites. A section from an uninfected feeder root is illustrated in Figure 2b.

Parasitized storage roots showed many so-called "stone cells." Dissection of these "cells" revealed that each contained a mature Meloidogyne female and egg mass. Following staining, these "cells" were determined to be a result of a host reaction in which a wound periderm was deposited about the nematode and associated egg mass (Fig. 2c). A section of uninfected storage root is illustrated for comparison (Fig. 2d). Although some eggs hatched, no larvae were observed to have escaped from this periderm-enclosed area. It is likely that this host reaction prohibits, or drastically limits, reinfection of the host plant by larvae hatched from eggs within "stone cells."

In the field, Meloidogyne-infected yam roots, when used as transplants or as propagation material, frequently outgrew infection and produced large roots relatively free from infection. This ability to overcome the root-knot disease is probably a direct result of the reduction of inoculum by the host's reaction to infection.

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REDUCTION OF POSTHARVEST BROWN ROT AND RHIZOPUS DECAY OF EASTERN PEACHES WITH HOT WATER

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Summary

Hot-water treatments reduced decay of peaches that had been inoculated with either Monilinia fructicola or Rhizopus stolonifer. Peaches, dipped for 7 minutes in 120° F, 3 minutes in 130°, or 2 minutes in 140° water, either 1 or 24 hours after inoculation, developed considerably less decay in 2 to 6 days at 70° than peaches dipped in 70° water. Hot-water treatments increased peach temperatures just under the skin about 31°. In the flesh near the pit, temperatures increased 15.3°, 6.7°, and 4.2° in peaches with 120°, 130°, or 140° dip treatments respectively. Tan surface mottle sometimes occurred when peaches were dipped in 140° water. None of the treatments caused off-flavor or affected ripening.

INTRODUCTION

Refrigeration during holding or transit is the most common method of delaying ripening and decay of produce. To supplement refrigeration, chemical treatments are often used to reduce decay. Both of these methods have weaknesses. Low temperatures effectively delay ripening, but usually only retard the growth of pathogenic microorganisms, and produce, when moved to higher temperatures, may decay rapidly. Most chemical treatments are toxic only to organisms on the surface of the product. When the decay-producing organism penetrates the produce, the chemical, therefore, is of no value. Chemicals may also leave unsightly residues on the surface of the produce and may cause off-flavors. Finally, residue tolerances in, or on, produce must be established by the Food and Drug Administration before a chemical can be used on produce destined for interstate trade.

Heat treatments to reduce postharvest decay also have been investigated, but the results of these tests have mostly been ignored or overlooked. In 1922 Fawcett showed that brown rot of lemons was prevented by dipping the fruit for 2 minutes in 115° to 120° F water (4). Additional studies on the hot-water treatment for lemons recently were reported by Klotz and DeWolfe (5). Hot water or vapor heat treatments also reduced Colletotrichum decay of papayas in studies conducted in Hawaii by Akamine, Akamine and Arisumi, Arisumi, and Shoji (1, 2, 3, 8). Hot air also has successfully reduced postharvest decays. In 1949 Kushman and Cooley showed that the development of black rot in sweetpotatoes was prevented by heating the roots in 110° air for 1 day before curing and storing (6). Since about 1958 the Australians have been conducting tests with hot air to reduce brown rot of peaches for canning. Tindale, Jenkins and Peggie showed that immature canning peaches could be ripened to full color at 35° C without development of brown rot. They cautioned, however, that particularly under high relative humidity rots due to Rhizopus sp. and Aspergillus sp. may develop (10). In later reports this group of workers considered that holding peaches for 1 day at 104° F, and then transferring them to 80° for ripening gave excellent control of all decays[1]. Miller, et al. (7) showed that the dormant spores of both Monilinia fructicola (Wint.) Honey and Rhizopus stolonifer (Fr.) Lind. held for 2 hours on potato-dextrose agar at 105° F did not germinate when they were transferred to 75°

Such studies suggested that short exposures to high temperatures may reduce postharvest decay of peaches destined for the fresh market. A preliminary report on hot-water treatments to reduce decay of peaches has been given (9).

MATERIALS AND METHODS

Peaches were obtained from the wholesale market in Washington, D. C. or from nearby orchards. Test peaches were sorted for uniform size and maturity and were free from decay, bruises, and other blemishes.

Fruit was artificially inoculated by dipping in a suspension of spores in beef-peptone broth containing 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate). Uninjured fruit was inocu-

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[1] Unpublished Departmental Papers, Horticultural Division, Department of Agriculture, Melbourne, Australia. Received November 1961.

lated with spores of the brown rot organism (Monilinia fructicola) while a shallow cut, resembling a finger nail injury, was made on each cheek of fruit inoculated with Rhizopus stolonifer. The suspension contained 20 to 30 Monilinia or 70 to 80 Rhizopus spores per low power field of a microscope.

After inoculation the peaches were held at 70° F and about 90% relative humidity until they were treated. Treatments consisted of dipping fruit and containers in a constant temperature water bath at 120° for 7 minutes, at 130° for 3 minutes or at 140° for 2 minutes. Some samples were held for about 1 hour and others 24 hours after inoculation before they were treated. Twenty peaches were treated at each temperature and treatment time in each replication. After treatment, fruit in the wet containers was held at 70° and 90% relative humidity. Decay development was compared with decay of fruit dipped in 70° water for 7 minutes (checks).

The percentage of naturally inoculated peaches that developed decay, with and without the hot-water treatments, also was determined in each replication.

Temperatures of representative peaches in each replication were taken just under the skin and in the flesh near the pit immediately before and after treatment.

RESULTS

Artificially Inoculated Peaches: Firm-ripe peaches, picked about 24 hours before the test, were used in six replications; they became soft-ripe in 2 to 4 days at 70° F.

When these peaches were inoculated about 1 hour before treatment, decay development during holding at 70° was consistently less in peaches dipped in hot water than in the checks which were dipped in 70° water (Table 1). Treated fruit held 2 days at 70° developed no Monilinia and only a slight amount of Rhizopus decay, whereas the check fruit developed a slight amount of Monilinia and a moderate amount of Rhizopus decay. After 4 days at 70° both the Monilinia- and Rhizopus-inoculated check fruit developed extensive decay. Each of the hot-water treatments reduced decay of Monilinia- and Rhizopus-inoculated fruit by at least 80%. The hot-water treatments also reduced decay in the Monilinia- and Rhizopus-inoculated fruit by a high percentage when fruit was held 6 days at 70°, about 3 days after they were soft-ripe.

In three replications, fruit was in transit or held at low temperatures at least 2 days before it was used in the tests. This fruit was firm-ripe when inoculated, and it became soft-ripe in 1 to 2 days at 70° F (Table 2).

When these peaches were inoculated with Monilinia or Rhizopus spores about 1 hour before the hot-water treatments decay reductions during the holding periods at 70° F were somewhat less than when peaches were treated more promptly after harvest (Tables 1 and 2). Nevertheless, when the fruit was soft-ripe (after 2 days at 70°) decay of the treated fruit usually was at least 50% less than the decay of the checks (Table 2). Even after 4 days at 70°, decay of Monilinia-inoculated fruit was considerably, and Rhizopus-inoculated fruit usually substantially, less than the checks.

Hot-water treatments 24 hours after inoculation reduced decay development at 70° F as effectively as when the fruit was treated about 1 hour after inoculation (Tables 1 and 2).

Much of the total decay that developed during 4 or 6 days at 70° F on fruit inoculated with Monilinia spores was due to Rhizopus contamination (Tables 1 and 2), particularly when the fruit was overripe.

Naturally Inoculated Peaches: Hot-water treatments also reduced Monilinia and Rhizopus decay of naturally inoculated peaches. When fruit became soft-ripe in 2 to 4 days usually both Monilinia and Rhizopus decay were considerably less following hot-water treatments than in the check fruit (Table 3). The hot-water treatments reduced decay of fruit treated about 48 hours after picking equally as well as fruit treated about 24 hours after picking.

The hot-water treatments did not always effectively reduce decay in peaches that became soft-ripe in 1 to 2 days (Table 4). Most of this decay was due to Rhizopus developing on bruised areas or overripe fruit.

Fruit Temperatures after Treatment: Dipping peaches in 120°, 130°, or 140° F water for 7, 3, or 2 minutes respectively increased the temperature just under the skin about the same (Table 5). Flesh temperatures near the pit were increased the most (15.3°) by the 7-minute dip in 120° water and the least (4.2°) by the 2-minute dip in 140° water.

Fruit dipped for 2 minutes in 140° F water sometimes developed a light tan skin mottle during holding at 70°. The injury did not develop on fruit treated in lower temperature water. The treatments did not affect either ripening or flavor of the fruit.

Table 1. Effect of water temperature and delayed treatment on percentage total decay of artificially inoculated firm-ripe peaches. Peaches became soft-ripe in 2 to 4 days.

Treatment			Monilinia inoculated			Rhizopus inoculated[a]		
Delay after inoculation (hours)	Water temperature (° F)	Dipping time (minutes)	days at 70° F			days at 70° F		
			2	4	6	2	4	6
1	70 (check)	7	2.2	70.8[b]	89.2[c]	27.8	84.4	87.2
	120	7	0.0	2.0[d]	30.9[e]	0.8	15.8	25.0
	130	3	0.0	8.3[e]	20.0[d]	0.0	9.7	26.7
	140	2	0.0	13.3[c]	24.2[c]	0.8	12.2	26.1
24	120	7	0.8	0.8	9.7[d]	4.5	7.8	20.6
	130	3	0.8	6.4	26.3[c]	3.3	11.8	39.7
	140	2	0.0	7.5	27.2[c]	2.5	18.9	43.3

[a] Infection due almost entirely to Rhizopus.

[b] About 15% of decay due to Rhizopus contamination.

[c] About 25 to 35% of decay due to Rhizopus contamination.

[d] About 40 to 50% of decay due to Rhizopus contamination.

[e] About 60 to 70% of decay due to Rhizopus contamination.

Table 2. Effect of water temperature and delayed treatment on percentage total decay of artificially inoculated firm-ripe peaches. Peaches became soft-ripe in 1 to 2 days.

Treatment			Monilinia inoculated		Rhizopus inoculated[a]	
Delay after inoculation (hours)	Water temperature (° F)	Dipping time (minutes)	days at 70° F		days at 70° F	
			2	4	2	4
1	70 (check)	7	12.8	87.7[b]	59.4	93.3
	120	7	4.4	41.6[c]	11.0	32.2
	130	3	7.8	58.9[c]	8.9	71.1
	140	2	2.3	38.9[a]	15.6	61.7
24	120	7	2.2	28.6[d]	18.3	50.0
	130	3	5.6	30.6[c]	23.9	51.7
	140	2	1.6	30.0[d]	21.6	72.8

[a] Infection due almost entirely to Rhizopus.

[b] Less than 10% due to Rhizopus contamination.

[c] About 40 to 50% due to Rhizopus contamination.

[d] About 65% due to Rhizopus contamination.

Table 3. Effect of water temperature and delayed treatment on percentage total decay of naturally inoculated firm-ripe peaches. Peaches became soft-ripe in 2 to 4 days.

Treatment			Monilinia			Rhizopus		
Delay after picking (hours)	Water temperature (° F)	Dipping time (minutes)	days at 70° F			days at 70° F		
			2	4	6	2	4	6
24	70 (check)	7	0.0	8.6	19.2	0.0	7.5	12.5
	120	7	0.0	0.0	1.7	0.0	0.8	5.8
	130	3	0.0	5.2	6.1	0.0	7.8	15.0
	140	2	0.0	1.7	3.7	0.0	2.8	4.2
48	120	7	0.0	0.0	1.7	0.0	0.0	0.8
	130	3	0.0	0.0	0.0	0.0	0.0	3.3
	140	2	0.8	1.7	1.7	0.0	0.0	9.2

Table 4. Effect of water temperature and delayed treatment on percentage total decay of naturally inoculated firm-ripe peaches. Peaches became soft-ripe in 2 to 4 days.

Treatment			Monilinia		Rhizopus	
Delay after picking (hours)	Water temperature (° F)	Dipping time (minutes)	days at 70° F 2	4	days at 70° F 2	4
48	70 (check)	7	11.7	22.9	0	3.3
	120	7	0	6.1	6.7	23.9
	130	3	0	4.5	0	20.5
	140	2	0	7.3	0	18.3
72	120	7	6.7	5.6	0	3.3
	130	3	0	0	0	8.3
	140	2	0	0	0	5.0

Table 5. Effect of hot water dips on peach flesh temperatures.

Temperature of water (° F)	Duration of dip (minutes)	Before dip[a] (° F)	After dip: under skin[b] (° F)	After dip: near pit[a] (° F)	Increase: under skin[b] (° F)	Increase: near pit[a] (° F)
120	7	76.3	109.9	91.6	33.6	15.3
130	3	78.2	108.3	84.9	30.1	6.7
140	2	78.7	108.6	82.9	29.9	4.2

[a] Mean 60 readings.
[b] Mean 120 readings.

DISCUSSION

Treatment of peaches with hot water is shown to be an extremely effective way to reduce postharvest decay due to Monilinia fructicola or Rhizopus stolonifer. Hot-water treatments have several advantages over chemical methods of decay reduction. The hot-water treatments leave no toxic or unsightly residues, and eliminate the problem of maintaining effective chemical concentrations in the treatment solution. An outstanding advantage of the hot-water treatment over chemical treatments is the destruction of the organisms even after they have entered the fruit. Incipient, as well as surface infections, therefore, may be eliminated or greatly reduced by these treatments.

During these studies, no attempt was made to isolate the containers of the treated peaches from those of the checks, or those containing peaches inoculated with Monilinia spores from those containing peaches inoculated with Rhizopus spores. Consequently, cross contamination from one container of peaches to another was probable. Rhizopus contamination was the chief offender. Even with this contamination, decay was much less in the treated peaches held for 2 to 4 days at 70° F after they were soft-ripe than in the untreated fruit.

The use of heat to control microorganisms is not a new idea, in fact it is one of the oldest methods known. Hot-water treatments have long been accepted as the best method to reduce incipient infections in seeds. Sterilization of soil by steam or hot water is customary. Milk is pasteurized by high temperatures. The possibility of using high temperatures to reduce decay of peaches is, therefore, reasonable.

In these tests the maximum increase in peach temperature in the flesh near the pit was about 15 degrees, an increase from 76.3° to 91.6° F. During commercial handling, peaches must be refrigerated to below 50° during holding or transit to delay ripening. If peaches were to be treated with hot water before holding or transit, cooling the fruit after treatment would require additional refrigeration. The hot-water treatment of peaches, therefore, may have advantages and possibilities for commercial use. Methods of commercial application, however, have yet to be developed.

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PHYSIOLOGIC SPECIALIZATION OF PUCCINIA GRAMINIS AVENAE IN COLOMBIA[1]

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Summary

Stem rust of oats is the limiting factor in oat production in Colombia. The appearance of new races has frustrated all efforts to produce resistant varieties. Seven hundred isolates and collections of Puccinia graminis avenae have been studied since 1959 and have been classified into 22 subraces consisting of 6 physiologic races. One subrace, 6C, has the capacity to attack all sources of resistance presently known.

INTRODUCTION

Although oat stem rust, Puccinia graminis avenae Eriks. & E. Henn., has probably existed in Colombia for many years, it was not until 1930 that it was reported for the first time on Avena sativa south of Bogotá (4). Subsequent studies showed that it was widespread not only in the Savanna of Bogotá but also in the state of Nariño, and the pathogen is now known to exist wherever cultivated or wild oats are found. Wild oats are a common weed in Colombian wheat fields.

In 1950 oat stem rust was recognized as one of the most serious diseases of the crop. The development and expansion in 1957 of the oat breeding program of the Cooperative Program between the Ministry of Agriculture of Colombia and The Rockefeller Foundation demonstrated that the magnitude of the problem was considerable, although the lines with Hajira-Joanette in their parentage were resistant or moderately resistant at this time.

The situation has progressively deteriorated since 1958. In 1959 the pathogen prevented the release of a local selection of C.I. 6969 (Canuck x Florida 167-Landhafer) that had been multiplied for distribution to farmers and reduced progress in the breeding program by eliminating hundreds of lines which had been initially resistant (20). This culminated in 1960 when all the material in the oat program became susceptible in the field and necessitated the temporary suspension of the crossing program because of lack of resistant parents (13, 14, 21).

Oats have never been an economically important crop in Colombia. Experiments have demonstrated, however, that in the high altitudes of Colombia oats have great possibilities for forage and grain. No other crop tested to date has shown as great a potential for forage production. If adapted varieties are used, oats can be planted twice a year, and under experimental conditions yields of 70 metric tons per hectare of fresh forage (14, 20) and of more than 5 metric tons per hectare of grain have been obtained.

MATERIALS AND METHODS

Studies on the identification of physiologic races of P. graminis avenae were initiated at the Tibaitatá experiment station, near Bogotá, in 1958. Because of the marked fluctuations in temperature in the greenhouse, the first year's data were not completely reliable and are not included in this study.

In late 1959, however, a "Floral Breeze" cooling system was installed in two sections of the greenhouse. This system is regulated thermostatically and lowers high temperatures but cannot increase low temperatures. To increase low temperatures, at least within a limited space, glass cabinets were built over the tables of the Floral Breeze cooled greenhouse, and the low temperatures were thermostatically regulated, with electric hair dryers used as heaters. By this means, temperatures in the glass cabinets could be maintained with a daily variation of 5° C.

In early 1960 three Percival growth cabinets were installed which permitted even more precise regulation of temperature, light, and to some degree relative humidity. Maximum temperature fluctuation within these cabinets is 3° C.

The graphs in Figure 1 show the temperature and humidity obtained in (a) the Floral Breeze cooled greenhouse, (b) the uncooled greenhouse, and (c) the growth cabinets.

Fourteen isolates of P. graminis avenae, which had previously been tested in the greenhouse, were studied simultaneously in different locations in the cooled greenhouse, in the glass cabinets, and at 18°, 24°, and 29° C in the three growth cabinets. The results indicated that the reactions given by the races incubated in the cooled greenhouse were the same as those in the growth cabinet at 18°, and that race identification made in the cooled greenhouse could be used with confidence.

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Seven hundred cultures, including isolates (88% of the total) and collections (12%), of Puccinia graminis avenae were studied in Floral Breeze cooled greenhouses during 1959, 1960, and 1961.

For the identification of physiologic races and subraces, the usual three standard differential varieties or their equivalents (1, 15, 16, 23) were used, as well as six of the more common supplementary varieties (19, 24, 25): Rodney (R. L. 2123), New Garry (C.I. 6662), Ag. 331 (C.I. 7144), Ag. 313 (C.I. 6909), Burnett (C.I. 6537), and Saia (C.I. 4639). In addition, the varieties Bond (C.I. 7004) and/or Gopher (C.I. 2027) were included in all tests as susceptible varieties. In some of the later tests the barley variety Funza and the wheat variety Einkorn (C.I. 2433) were also included.

The collections of oat stem rust have come primarily from cultivated oats (Avena sativa), with some from wild oats (A. fatua), and limited numbers from orchardgrass (Dactylis glomerata) and English ryegrass (Lolium perenne). A high percentage (88%) of the collections studied originated from single pustules isolated from plants of the C.I. 6969 selection exposed to naturally occurring infection at Tibaitatá for 24 hours and then placed in moist chambers. Such collections were made daily.

Inoculations were always made by spraying at 10 pounds of pressue with a "Gast" portable air compressor connected by a rubber tube to a nozzle from a "De Vilbis" No. 15 atomizer. The spores were mixed in a solution of 0.1% agar.

Four differential varieties were planted per 10.5-cm pot, the seeds of each variety being placed at a maximum distance from the other three. When some care was used, there was no danger of mixing the varieties. The soils used for planting consisted of one part coarse sand and two parts silt-loam soil. A 10-hour day length at 1400 to 1600 foot candles was used in the growth cabinets.

Eight days after planting, differential varieties were inoculated in metal, circular-shaped moist chambers lined with thick, moist cloth to assure a permanent relative humidity of 100%. The average temperature in the moist chambers during incubation was 15° C.

Inoculated plants were held in the moist chambers for 15 to 16 hours, then slowly dried for 4 hours, and afterward transferred to the Floral Breeze cooled greenhouses where they remained until notes were taken on infection types. This method is similar to that described by Rowell, et al. (22) and was highly satisfactory for obtaining excellent infection with oat stem rust under Colombian conditions.

Infection types were noted 15 to 17 days after inoculation of the differentials in accordance with the description given by Bailey (1), Levine and Smith (15), and Newton and Johnson (16).

RESULTS

The classes of reaction produced by the 700 collections and isolates of P. graminis avenae on the standard differential varieties plus the supplementary differentials and their classification as races and subraces are given in Figure 2. The differentials have been arranged in order according to their known genotypes (19) and the races and subraces have usually been placed in order according to their virulence. The respective percentages of the total are given for each race and subrace.

Orjuela (17) prepared a tentative classification of races and subraces of P. graminis avenae in 1960-1961 based on the reactions produced by 148 isolates, or collections, or both. Using later information, we have simplified this classification and reduced the number of subraces. Subraces which represented only one or two collections were not included because of the possibility of escape, or misreading, and a few were omitted because of impurities found in the differential varieties.

In addition, on the basis of the class of reaction produced on the standard and supplementary differentials, a key has been prepared for the identification of races and subraces (Table 1). The key includes the three standard differentials, Minrus, Richland, and Jostrain, for the separation of races and the supplemental varieties New Garry and Saia for the differentiation of the majority of subraces. Rodney and Burnett are necessary only to separate race 7D. Except for subraces 4D, 6D, and 13D, Ag. 331 and Ag. 313 react identically to Richland, and similarly to New Garry in all cases although the infection type is more susceptible with this

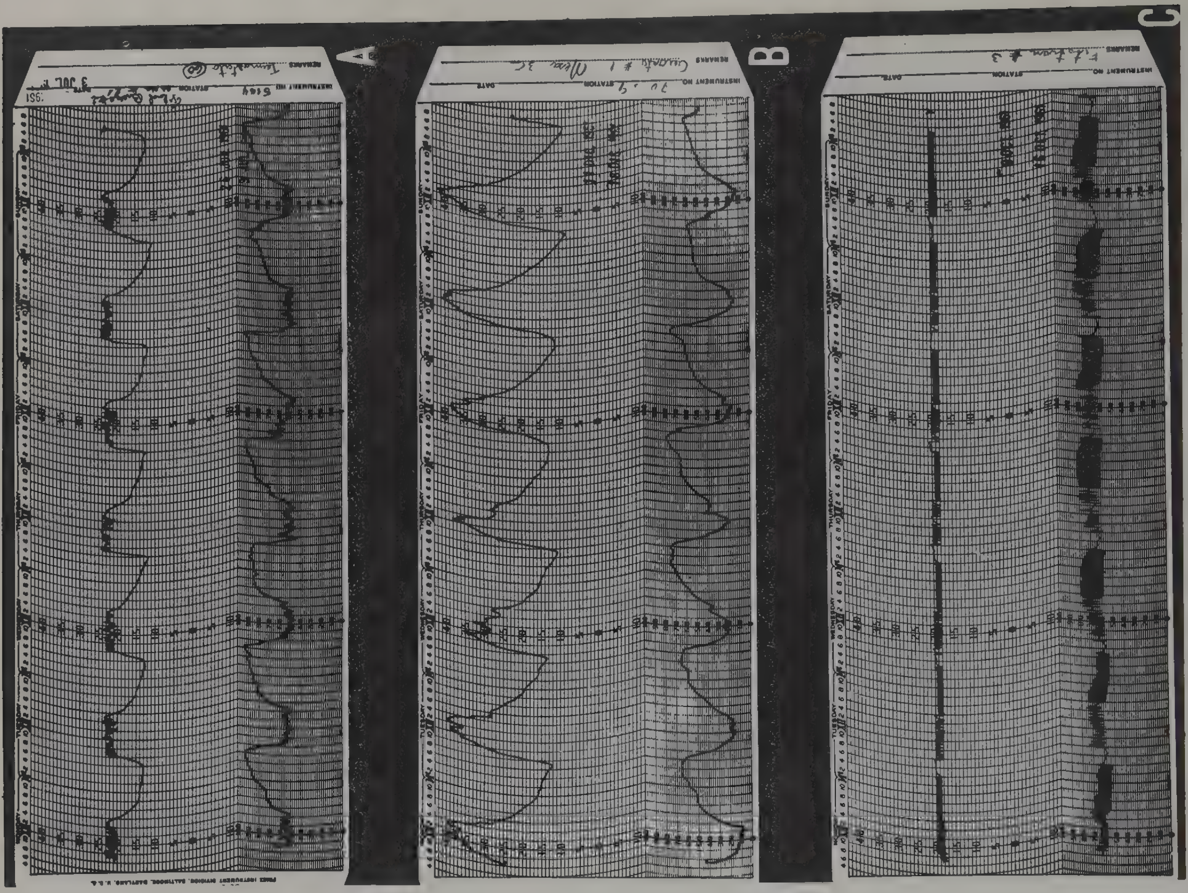

FIGURE 1.

Differentials	C.I. No.	Probable genotype	Reaction to races and subraces																					
			3			12			7				4				13				6			
			B	C	D	A	B	C	A^{a}	B	C	D	A^{a}	B	C	D	A^{a}	B	C	D	A^{a}	B	C	D
Richland	787	A	○	○	○	○	○	○	○	○	○	○	●	●	●	●	●	●	●	●	●	●	●	●
Rodney	(R.L. 2123)	BC	●	●	●	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●	●	●	●
Minrus	2144	D	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Jostrain	2660	E	○	○	○	◐	◐	◐	●	●	●	●	○	○	○	○	◐	◐	◐	◐	●	●	●	●
Ag. 331^{b}	7144	AD	○	○	○	○	○	○	○	○	○	○	●	●	●	○	●	●	●	○	●	●	●	○
New Garry	6662	ABC	○	○	○	○	○	○	○	○	○	○	●	●	●	○	●	●	●	○	●	●	●	○
Burnett	6537	BCD	●	●	●	●	●	●	●	●	●	○	●	●	●	●	●	●	●	●	●	●	●	●
Ag. 313^{b}	6909	ABCD	○	○	○	○	○	○	○	○	○	○	●	●	●	○	●	●	●	○	●	●	●	○
Saia	4639	?	●	◐	○	◐	●	○	○	●	◐	●	○	◐	●	○	○	◐	●	◐	○	◐	●	◐
Percentage of^{c}		Subraces	0.71	5.43	4.43	13.00	3.15	0.71	1.71	1.57	3.15	0.71	8.71	5.15	0.71	1.00	1.57	27.60	6.14	0.86	1.29	5.71	6.43	0.86
		Races	10.57			16.86			7.14				15.57				35.57				14.29			

a = Previously described subraces

b = [Landhafer x (Mindo x Hajira-Joanette)] x Andrew

c = Based on 700 isolates and/or collections

○ = Resistant (infection types: 0, 0;, 1, 2)

● = Susceptible (infection types: 3, 4)

◐ = Mesothetic (infection types: X)

(See legends on opposite page)

FIGURE 2.

Table 1. Key for the identification of physiologic races and subraces of *Puccinia graminis avenae* on the basis of their pathogenicity on standard differential and supplementary varieties of *Avena* spp.

Reaction of differential varieties and supplementary varieties	No. of physiologic race and subrace
Minrus Susceptible	
Richland Resistant	
Jostrain Resistant	
New Garry Resistant	
Saia Resistant	3D
Saia Mesothetic	3C
Saia Susceptible	3B
Jostrain Mesothetic	
New Garry Resistant	
Saia Resistant	12C
Saia Mesothetic	12A
Saia Susceptible	12B
Jostrain Susceptible	
Rodney Resistant	
New Garry Resistant	
Burnett Resistant	
Saia Susceptible	7D
Rodney Susceptible	
New Garry Resistant	
Burnett Susceptible	
Saia Resistant	7A
Saia Mesothetic	7C
Saia Susceptible	7B
Richland Susceptible	
Jostrain Resistant	
New Garry Resistant	
Saia Resistant	4D
New Garry Susceptible	
Saia Resistant	4A
Saia Mesothetic	4B
Saia Susceptible	4C
Jostrain Mesothetic	
New Garry Resistant	
Saia Mesothetic	13D
New Garry Susceptible	
Saia Resistant	13A
Saia Mesothetic	13B
Saia Susceptible	13C
Jostrain Susceptible	
New Garry Resistant	
Saia Mesothetic	6D
New Garry Susceptible	
Saia Resistant	6A
Saia Mesothetic	6B
Saia Susceptible	6C

variety (4 instead of 3 for Ag. 331 and Ag. 313).

Twenty-two distinct subraces have been found, consisting of six distinct physiologic races of *P. graminis avenae*. Four of these subraces, 4A, 6A, 7A, and 13A, have been described in other countries (2, 3, 10, 11, 12, 18, 23, 24), but the remainder are new. Roberts and Stewart (19) recently reviewed the descriptions of the 19 races and subraces of oat stem rust that have occurred in North America up to 1960 including 4A, 6A, 7A, and 13A.

Subrace 3B of Colombia is probably identical with subrace 3A of Sweden (11, 12), but until the reaction of 3A on the supplemental differentials, except Rodney, is known the identity cannot be established with precision.

Subrace 13B was discovered in Colombia in October 1958, from collections made from oats at Tibaitatá, and it was reidentified at various times in 1959, 1960, and 1961 under controlled temperature conditions. With the exception of 12A, 12B, and 12C, all the other subraces were found in 1959 and tentatively described in 1960-1961 (17).

From five collections made from wild oats subraces 4B, 6C, and 13B were identified; from orchardgrass, subrace 13B; and from English ryegrass, subraces 4A, 4B, 6B, 6C, and 13D. Some of the most virulent subraces have been isolated from these grasses.

FIGURE 1. Temperature and humidity obtained in the Floral Breeze cooled greenhouse (A), the uncooled greenhouse (B), and the Percival growth cabinets (C).

FIGURE 2. Reactions produced by isolates and/or collection of *Puccinia graminis avenae* on the standard and supplemental differential varieties of *avenae* spp. and their classification as races and subraces.

DISCUSSION

The fact that the majority of the isolates (88%) were trapped and multiplied on the C.I. 6969 selection, a line which is resistant to many of the Colombian races, probably explains why only races and subraces virulent to Minrus have been isolated. An interesting study will be the collections from Gopher or Bond and/or collections multiplied on these varieties to discover which of the races and subraces avirulent on Minrus, such as 1, 2, 5, 8, 9, 10, and 11, exist in Colombia. Nevertheless, the use of C.I. 6969 to trap spores and multiply the isolates and collections has been of great advantage because it has permitted the isolation, identification, and study of a very high proportion of the most virulent races and subraces. These are, of course, the races of most interest to a breeding program.

The extreme physiologic variation of P. graminis avenae in a country where oats are not cultivated is surprising. The isolation of subraces as highly pathogenic as 4A, 4B, 13B and, particularly, 6C from wild oats and certain grasses suggests that these are important hosts which can permanently lodge virulent subraces and can possibly facilitate the formation of new races. Wild oats are a very common weed in wheat and other crops and often persist for some time after harvest. This weed has existed in Colombia perhaps as long as wheat, orchardgrass, and English ryegrass, and it undoubtedly was present long before the initiation of an oat breeding program. In all probability, the subraces isolated from these hosts also existed long before the initiation of the breeding program.

Little is known about events in the repeating stage of P. graminis avenae when it is constantly passing between oat and grass hosts. Possibly a progressive evolution of virulence has occurred through natural selection in the absence of large populations of oats. If the species of Berberis existing in Colombia plays some part in the life cycle of the pathogen, the alternation between Berberis and oats and other grasses magnifies the potential for virulence still further.

Subraces simultaneously virulent on Rodney and Garry have been found in Sweden (10, 11, 12), Canada (3, 7, 8, 9), and in the northern United States (19, 23, 24, 25). Subraces of still greater virulence from a high, cool tropical climate are reported here. The fact that Sweden, Canada, and the northern part of the United States generally have a cool climate during the growing season may indicate that a combination of relatively low temperatures and suitable hosts favors variation and accumulation of virulence in the fungus.

Subrace 6C is the most virulent subrace described in this paper. It attacks all the known sources of resistance, including C.I. 4020, C.I. 4023, and C.I. 4539, which have been found resistant to 6A by Green, Johnson, and Welsh (8) in Canada, and the generally highly resistant diploid Saia. It would appear that 6C is the most virulent race known in the world. The possible consequences of introducing this subrace into other temperate regions, particularly North America where the oat industry is of considerable economic importance, should not be underestimated.

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THE COLOMBIAN MINISTRY OF AGRICULTURE AND THE COLOMBIAN AGRICULTURAL PROGRAM OF THE ROCKEFELLER FOUNDATION

NEMATOPHAGUS FUNGI RECOVERED AROUND HIGHLANDS, NORTH CAROLINA[1]

William A. Feder[2]

The recovery of nematode-capturing predaceous fungi has been reported from many habitats in many parts of the world. The ecology of these fungi and the factors affecting their distribution are little understood. A project has been undertaken to collect these fungi from various habitats at various locations, ranging from the lowlands of Florida and Georgia to the foothill and mountain areas of the eastern seaboard. Fungi collected from these places can then be compared with relation to growth-temperature optima, nutrition, and nematode-capturing ability. This paper lists those organisms collected during the summer of 1961 in the vicinity of the Highlands Biological Station, Highlands, North Carolina.

MATERIALS AND METHODS

Collections in the Highlands area were taken at 46 locations. Material from these collections was divided into 200 subsamples plated on Difco cornmeal agar in plastic Petri dishes for recognition and subsequent isolation and identification of the nematode-capturing predaceous fungi in the collections. An additional 200 subsamples from the original collections were plated 4 months later and examined to insure a maximum recovery of predaceous fungi. Collections from mosses and rotting leaves and wood were made at elevations of 1500 to 6500 feet. The Phycomycetous fungi were identified *in situ* in the plates in which they were discovered. Positive identification of the predaceous Hyphomycetes was insured by exposing cultures from single-spore isolates of these fungi to blocks of agar containing a viable mixed culture of the nematode *Aphelenchoides sacchari* Hooper and the fungus *Penicillium digitatum* Sacc. The presence of this nematode in the culture caused a predaceous fungus to form its characteristic organs of capture and permit ready identification.

RESULTS AND DISCUSSION

Table 1 shows the various fungi recovered, the elevation of the collection site, and the frequency of recovery of the various species. The area yielded a wide variety of forms in all three groups. Of interest is the fact that certain of these fungi such as *Dactylella ellipsospora*, *Arthrobotrys oligospora*, *Harposporium anguillulae*, and *Acrostalagmus obovatus* recovered from the high, moist elevations around Highlands are also consistently recovered from the low, hot, relatively dry, sandy Florida citrus grove soils. The moss cushions were a good source of nematode-capturing Phycomycetes, probably because of the high humidity within these cushions.

Exploration of the Highlands, North Carolina area has scarcely begun. The natural river gorges have not been searched for nematode-capturing fungi. These gorges provide a wide variety of habitats within a limited area and an excellent place for a proper ecological study of these fungi. Also, a study of the distribution of these fungi up the slope from the Piedmont of Georgia to the South Appalachians should be made. Finally, a study should be undertaken of the types of nematodes used as prey by these fungi to relate nematode and nematode-capturing fungus distributions. This type of study has never been made and would be a most useful contribution to an understanding of the distribution of these fungi.

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[1] These collections were made possible by a Grant G-4977 from the National Science Foundation to the Highlands Biological Station, Highlands, North Carolina and were carried out while the author was in residence at the Highlands Biological Station.

[2] Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

Table 1.

Name	Frequency of recovery	Elevation of recovery location (feet)	Air temp. at recovery time (° F)
Phycomycetes			
Acaulopage acanthospora Drechsler	1	4000-4500	68
A. stenospora Drechsler	1	4000-4500	68
Cochlonema megalosomum Drechsler	1	2500-3000	68
Protascus subliformis Dangeard	1	4000-4500	64
Stylopage cephalote Drechsler	2	4000-4500	64
S. grandis Duddington	1	2500-3000	70
S. hadra Drechsler	2	3000-3500	84
S. rhabdoides Drechsler	1	3000-3500	84
Moniliales			
Acrostalagmus gonoides Drechsler	1	4000-4500	70
A. obovatus Drechsler	1	4000-4500	100
Arthrobotrys dactyloides Drechsler	1	3500-4000	63
A. oligospora Fresenius	5	2500-4500	63-80
Dactylaria thaumasia Drechsler	2	4000-4500	70
Dactylella bembicoides Drechsler	2	2500-3000	70
D. cionopaga Drechsler	2	5000-5500	76
D. doedycoides Drechsler	2	6000-6500	68
D. ellipsospora Grove	2	2500-3000	63
Harposporium anguillulae Lohde	4	1500-4500	68-80
H. oxycoracrum Drechsler	2	2500-3000	75
Spicaria coccospora Drechsler	1	3000-3500	63
Basidiomycetes			
Nematoctonus leptosporus Drechsler	1	4000-4500	65
N. tylosporus Drechsler	2	3000-3500	84

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, ORLANDO, FLORIDA

A NEW PHYTOPHTHORA FRUIT ROT OF ANNONA SQUAMOSA FROM INDIA[1]

Vasant Gurunath Rao, M. K. Desai, and N. B. Kulkarni[2]

Summary

A new Phytophthora fruit rot of custard apples (Annona squamosa) incited by Phytophthora parasitica Dast. var. macrospora Ashby is described for the first time from India. The pathogen persists through mummies and seed.

INTRODUCTION

Annona squamosa, locally known as Sitaphal, is a small naturalized tree and is found growing wild in India. The fruit is also known as "sugar apple" because of its top quality, taste and high content of sugar and is very popular in this country. A new disease resulting in rotting and dropping of mature and immature fruits was noticed for the first time in Poona, India in July 1957. Isolations from infected fruits repeatedly yielded a species of Phytophthora in pure culture. Since this appeared to be the first record for India, a detailed investigation was undertaken on the etiology of the disease and the pathogen, a brief account of which is presented in this note.

SYMPTOMS

The characteristic symptoms of the disease are the appearance of water-soaked areas in early stages, turning brownish purple to blackish on young, green and succulent fruits which later enlarge and cover the entire surface. Such affected fruits dry up, shrivel, become hard, and fall to the ground. Young as well as mature fruits are affected, resulting in severe fruit drop. In advanced stage of attack, fruits become light in weight, rind turns brownish in color, and seeds within shrivel, become hollow and lose weight. Fruit symptoms are shown in Figure 1. No other part of the tree was affected. The disease is favored by humid weather and drizzling rainfall and is common during the monsoon months (June to September).

PATHOGENICITY

The fungus was readily obtained in pure condition from the seeds and rind of affected fruits obtained directly from the tree or from fruits on the ground. The pathogenicity of the isolated species of Phytophthora was proved on healthy green fruits of custard apple with and without injuries under artificial inoculation experiments. Light purple water-soaked patches developed over the inoculated areas within 72 hours under moist chamber conditions. The fungus made good, fluffy, cottony-white, filamentous growth on the inoculated fruits and covered the entire surface of the fruits by the end of 15 days, causing softening and rotting. Controls remained healthy and intact. Half-ripened fruits appeared more susceptible than green fruits.

HOST RANGE

Besides custard apple, the fungus readily infected fruits of Carica papaya, Pyrus malus, Lycopersicum esculentum, Solanum melongena, Capsicum frutescens, Musa paradisiaca, Solanum tuberosum, Ficus carica, Cucumis sativus, Psidium guajava, Citrus medica var. acida, Citrus aurantium var. sinensis, and Hibiscus esculentus. It also produced damping-off of seedlings in Phaseolus vulgaris, Pisum sativum, Gossypium hirsutum, Ricinus communis,

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[1] Part of the work submitted by the senior author to the University of Poona, in fulfillment of the requirements for the degree of Master of Science (Agriculture).

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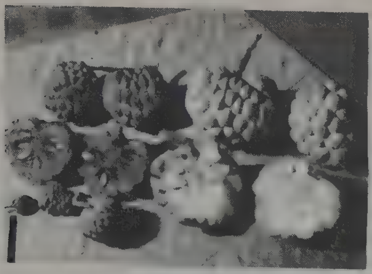

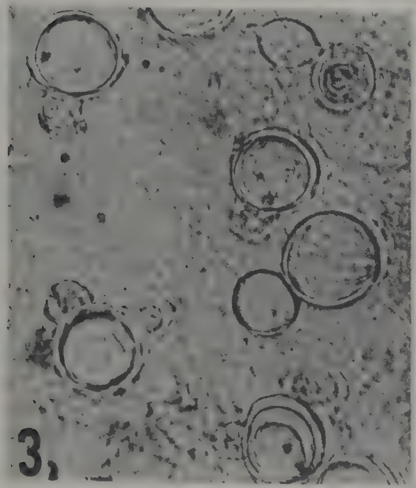

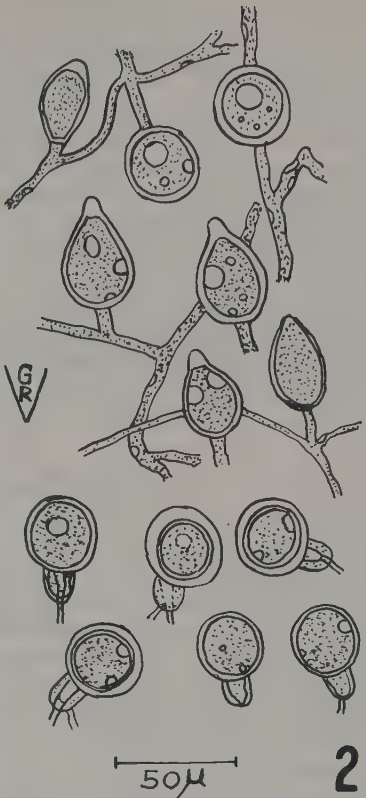

FIGURE 1. Diseased (left) and healthy (right) fruits of custard apple.

FIGURE 2. Camera lucida drawings of Phytophthora parasitica var. macrospora showing sporangia, chlamydospores, and amphigynous oospores.

FIGURE 3. Photomicrograph of amphigynous oospores on oatmeal agar in two-month-old culture. Approx. X 563.

Sesamum indicum, Solanum melongena, and Vinca rosea under artificial inoculation experiments. Brown rot was also noticed in inoculated young leaves of Bryophyllum pinnatum and phylloclades of Cactus species.

MORPHOLOGY OF THE FUNGUS

The fungus produced abundant papillate, sporangia and spherical chlamydospores on host and in culture media, especially of the non-synthetic types. On the host the sporangia measure 28.8 - 53.4 (40.4)μ in length and 15.4 - 24.8 (19.2)μ in breadth and chlamydospores 19 - 38.2 (27.7)μ in diameter (Fig. 2).

The fungus produced abundant amphigynous oospores in 2-month-old cultures on oatmeal agar, measuring 16 - 25 (21)μ in diameter (Fig. 3).

CULTURAL CHARACTERS

The fungus made good growth with abundant, fluffy, filamentous cottony-white, aerial mycelium on oatmeal, cornmeal, lima bean, gram meal and steamed rice agars and also on carrot extract (2%), papaya extract (2%), Sitaphal extract (2%) agars with abundant production of typically papillate, pear-shaped sporangia at the end of 6 days and spherical chlamydospores in older cultures. It made scanty growth on synthetic media like Kirchhoff's agar, Dox's agar, Coons' agar, Czapek's agar, and Richard's agar.

On oatmeal agar the fungus grew best at 28° to 30° C with minimum at 15° and maximum at 37°.

IDENTITY OF THE FUNGUS

Taxonomy of the genus Phytophthora de Bary has been described by Ashby (1), Tucker (3), and recently by Waterhouse (5). Emphasis has been placed on sporangial measurements, morphological character, cultural behavior, temperature-relations, and host range in the determination of species. Ashby (1), working with Phytophthora parasitica Dast., distinguished two strains, namely microspora and macrospora on the basis of oospore measurement.

On the basis of these characters, the present fungus agrees with Phytophthora parasitica Dast. var. macrospora Ashby. The higher temperature-relation of the present fungus, namely 15°, 28° to 30°, and 37° distinguishes it from Phytophthora palmivora Butler as described by Tucker (3) and Uppal and Kamat (4).

A similar fruit rot of Annona squamosa was reported by Purss (2) from Queensland and attributed to Phytophthora palmivora Butler.

The occurrence of Phytophthora parasitica var. macrospora on Annona squamosa is a new record from India.

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DIFFERENTIATION OF TOBACCO RINGSPOT AND TOMATO RINGSPOT VIRUSES BY EXPERIMENTAL HOST REACTIONS

D. M. McLean[1]

Abstract

Greenhouse studies were conducted to determine plant species which show a differential symptom reaction to tobacco ringspot virus from tomato ringspot virus after mechanical inoculations. Of 43 different species tested, 11 showed differential reactions to one or the other virus in the following suggested order of sensitivity: sesame, lima bean, snapdragon, spinach, watermelon, eggplant, Phacelia campanularia, Gomphrena globosa, petunia, Chenopodium amaranticolor, and Gypsophila elegans. Typical symptoms were not always expressed after repeated inoculations with tomato ringspot virus and symptoms were masked in certain species in a glasshouse at temperatures above 85° F. Symptoms of tobacco ringspot virus were readily expressed up to 95° but were frequently masked in older plants.

INTRODUCTION

Tobacco ringspot virus (TRSV) and tomato ringspot virus (TomRSV) are serologically unrelated (6, 22), and infection by one does not cross-protect against the other (22). Each virus has a very broad experimental host range, often causing nearly similar symptoms in certain suscepts (18). TRSV occurs naturally in certain vegetables, mostly in the Compositae, Cucurbitaceae, Leguminosae, Malvaceae, and Solanaceae (8, 12, 14, 15, 16), whereas TomRSV occurs mostly on ornamentals and woody or semi-woody plants (2, 4, 9, 10, 13, 21) and is rarely observed on vegetables. Both viruses are transmitted by nematodes (Xiphinema spp.) (3, 6, 7).

TomRSV has not been reported from Texas, but TRSV is ubiquitous in the Lower Rio Grande Valley (14, 15, 16). Disease symptoms in some cucurbits in the Lower Rio Grande Valley sometimes resembled those incited by TomRSV (16). Since both viruses cause somewhat similar symptoms in the index species used in earlier survey studies (4, 13, 16, 18) TomRSV, if present, was not detected in the symptom syndrome. The objective of the present greenhouse study was to determine index species which would show differential symptom reactions to TRSV and TomRSV. This paper reports several species affected by one virus and not the other.

MATERIALS AND METHODS

TomRSV (AC-13, American Type Culture) was maintained on Nicotiana tabacum 'Kentucky Burley 16' with frequent transfers to cucumber and cowpea. TRSV, shown to be serologically related to Steere's AC-174 (14), was isolated from a common field infection. Mechanical inoculations were made with the aid of new 6-inch wooden pot labels, sterile cheesecloth pads, or with cotton-covered toothpicks dipped in freshly expressed sap from infected cucumbers or cowpeas and stroked lightly over leaves of test plants previously dusted with carborundum 400. Temperatures were maintained at 65° to 85° F. The inoculation potential of crude virus extract was not enhanced by dilution with 0.1 M K_2HPO_4 neutral buffer.

RESULTS

The test species inoculated and their reaction to each virus are presented in Tables 1, 2, and 3. Several species reacted weakly to one virus and expressed no symptoms of the other. Others expressed nearly similar symptoms of each virus. Species in these two categories were considered poor test indicators for differentiating TRSV from TomRSV. Certain species either expressed symptoms of only one virus or symptoms so distinctly different as to characterize infection of one virus from infection by the other. These were useful as indicator test plants to differentiate TRSV from TomRSV. In this category are listed the following species

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Table 1. Species with nearly similar symptoms after inoculation with TRSV and TomRSV.

Test species	Symptoms	
	TomRSV	TRSV
Gossypium hirsutum (13)[a] Figure 1		
'Stoneville 7A'	epinasty with purple zig-zag lines on inoc. cotyledons, systemic necrotic flecks and vein necrosis in terminal leaves, interveinal chlorosis, leaf distortion, chlorotic spots, recovery	necrotic spots on inoc. cotyledons, not systemic
'Acala 4-42'	do.	do.
'Deltapine 106'	do.	do.
'Wescot'	do.	do.
Datura stramonium (2, 4, 6, 13)	etched concentric-necrotic rings on inoc. leaves, systemic chlorotic spots becoming necrotic, recovery	faint chlorotic spots on inoc. leaves (not so pronounced as TomRSV), recovery with interveinal chlorosis
D. metel	do.	do.
Physalis peruviana	vein clearing, mild mottle, rugosity, distortion of terminals, recovery	same as TomRSV
Nicotiana tabacum (4, 10, 13, 18, 19)		
'Kentucky Burley 16'	small concentric lesions with necrotic borders on portions of inoc. leaves, systemic etched zig-zag patterns, recovery	systemic chlorotic spotting, zig-zag patterns, ringspots, recovery
N. glutinosa (2, 6, 10, 18)	systemic chlorotic ringspots becoming necrotic, etched patterns, more severe and quicker response than TRSV, difficult to inoculate	small systemic chlorotic to necrotic ringspots, difficult to inoculate
Cucumis sativus (2, 4, 6, 10, 13, 19)		
'National Pickling'	chlorotic spots on inoc. cotyledons, mild vein clearing and systemic mottle, leaf margins curled downward, rugosity, chlorotic spotting not pronounced, recovery	chlorotic spots on inoc. cotyledons, systemic chlorotic flecks to pronounced rings and spots, recovery
C. melo		
'PMR 45'	chlorotic spots on inoc. leaves, vein banding and feathering in new growth, mild mottle, recovery	systemic chlorotic spotting on new growth after inoc. cotyledons
'Rio Gold'	do.	do.
'Wescan'	do.	do.
Cucurbita pepo		
'Prolific Straightneck'	necrotic lesion with dark green borders on inoc. senescent cotyledons, systemic chlorotic spots to blotches in new growth	dark green to chlorotic spots on inoc. cotyledons, systemic chlorotic spots
'Tatume'	distinct chlorotic spots with greenish borders on inoc. cotyledons, systemic chlorotic spots and rings in new growth, vein necrosis, large necrotic blotches in older leaves	dark green to chlorotic spots on inoc. cotyledons, systemic chlorotic spots, difficult to inoculate, shows some field resistance
Beta vulgaris (10, 13, 19)		
Sugarbeet	chlorotic to necrotic spots on inoc. cotyledons, mild systemic spotting and mottling, recovery	chlorotic spots on inoc. cotyledons, mild systemic chlorotic spots, slight rugosity, recovery
Vigna sinensis (2, 10, 13, 18)		
'Blackeye'	chlorotic spots on inoc. primary leaves sometimes becoming reddish-necrotic, slower reaction and not so severe as TRSV	solid reddish lesions on inoc. primary leaves, systemic terminal necrosis with necrotic spots, lethal
Crotalaria juncea	twisting of terminals, wilting, systemic vein necrosis, leaf abscission, necrotic stem streaking, sometimes more rapid and complete recovery than TRSV	chlorotic spots on inoc. cotyledons, systemic terminal necrosis often lethal in young plants, chlorotic spotting, chlorosis, sometimes recovery
Phaseolus vulgaris (6, 10, 13, 18)		
'Black Valentine'	chlorotic spotting on inoc. primary leaves, systemic rugosity and twisting of terminals, less severe than TRSV	chlorotic spotting on inoc. primary leaves, terminal necrosis and rugosity
Zinnia elegans (2, 4, 10, 13, 19)	vein clearing in new growth, epinasty of terminal leaves, milder and slower systemic than TRSV, recovery	vein clearing in new growth, pronounced epinasty, recovery
Hibiscus esculentus	sometimes faint chlorotic spots on inoc. cotyledons, not systemic	chlorotic spots on inoc. cotyledons, systemic chlorotic spotting, slight leaf crinkle, mottle, partial recovery, chlorotic spots on fruits (natural field infection)
Helianthus annuus (10, 13, 19)	mild systemic chlorotic spots in new growth, recovery	chlorotic spots on inoc. leaves, systemic yellow venation in terminal leaves, chlorotic rings
Cyamopsis tetragonoloba (13)	irregular necrotic rings on inoc. cotyledons, vein clearing, terminal necrosis (sometimes lethal), recovery, slower reaction than TRSV	same as TomRSV but more severe, often lethal in young plants
Chenopodium murale	no reaction on inoc. leaves, systemic chlorotic spotting in new growth	necrotic spots on inoc. leaves, systemic terminal necrosis with chlorotic specks, recovery

[a] Numbers in parentheses indicate literature references to species previously tested by others.

Table 2. Species showing indistinct symptoms after inoculation with TRSV and TomRSV.

Test species	Symptoms	
	TomRSV	TRSV
Gilia capitata (4, 19)	-[a]	reddish twisted leaves, symptoms indistinct
Eschscholzia californica (4, 19)	-	sometimes yellow crown leaves with reddish margins, symptoms indistinct
Portulacca grandiflora (4, 19)	-	chlorotic spotting in terminal growth, production of small leaves, stunting
Lychnis sp.	-	irregular chlorotic blotches in terminal leaves, general chlorosis
Sedum acre (19)	-	-
Dianthus plumarius (4, 19)	-	faint chlorotic ringspots, general chlorosis, symptoms indistinct
Phlox drummondii (4, 10, 19)	faint chlorotic rings in diffuse light, leaf crinkle, wavy margins, severe rugosity	-
Tropaeolum majus (4, 19)	-	mild ringspotting, interveinal chlorosis, leaf margins curled upward, bud proliferation
Lycopersicon esculentum (4, 6, 10, 13, 18, 21)		
'Rutgers'	irregular target necrotic flecks resembling Stemphylium infections on inoc. leaves, pronounced epinasty, systemic vein necrosis and sinuous necrotic lines, resembles zinc deficiency, difficult to inoculate	-
'Chico'	do.	-
'Rio Grande'	do.	-
Arachis hypogaea		
'Spantex'	irregular necrotic blotches with dark centers on inoc. leaves, affected leaves becoming chlorotic, not systemic	-
Vitis candicans (13) (seedlings)	-	-
Crambe abyssinica	-	-
Lactuca sativa var. *longifolia* (13)		
'Cos' (Romaine)	-	-

[a]No reaction.

Table 3. Species with distinct and differential response to inoculation with TRSV and TomRSV.

Test species	Symptoms	
	TomRSV	TRSV
Sesamum indicum	faint mottling in new growth, twisting of terminal leaves, diffuse systemic chlorosis and vein yellowing, distinct recovery	distinct necrotic spots on inoc. leaves, systemic necrosis, lethal in young plants
Phaseolus limensis		
'Thoroughgreen'	-	necrotic blotches with light centers on inoc. primary leaves, abscission of affected leaves, usually no systemic symptoms, hypersensitive
Antirrhinum majus (2, 4, 21)	-	chlorotic spots becoming necrotic on inoc. leaves, wilting, terminal necrosis, necrotic streaks on stems
Spinacia oleracea (4, 13, 19)		
'Early Hybrid 7'	-	chlorotic stippling on inoc. leaves becoming systemic, stunting, diffuse chlorosis
Citrullus vulgaris (13)		
'Sugar Baby'	-	systemic chlorotic stippling in new growth
'Charleston Gray'	-	do.
'Blackstone'	-	do.
Solanum melongena	necrotic ringspot on inoc. leaves, systemic chlorotic ringspots, vein clearing and necrosis, stunting, recovery	systemic chlorotic spots becoming calico mottled in 3-4 wk., general yellowing (yellows), no recovery
Phacelia campanularia (19)	-	systemic terminal necrosis, lethal in young plants
Gomphrena globosa (2, 5, 13)	necrotic spots with reddish borders on inoc. leaves, systemic chlorotic spots in new growth, rugosity	faint diffuse chlorotic spots on inoc. leaves, systemic chlorotic spots in new growth, rugosity
Petunia hybrida (4, 6, 13)		
'Celestial Rose'	small necrotic rings on inoc. leaves, systemic pin-point necrotic specks, vein clearing, recovery	faint chlorotic ringspotting, general chlorosis. slower reaction than TomRSV, recovery
Chenopodium amaranticolor (6, 13)	pin-point chlorotic spots on inoc. leaves soon becoming systemic with twist-of terminals and necrosis	pin-point chlorotic spots on inoc. leaves, slower systemic reaction than TomRSV
Gypsophila elegans (2, 21)	chlorotic spots on inoc. leaves, systemic small chlorotic rings, distortion of flower shoots, recovery in some plants	wavy leaf margins, stunting, rugosity, recovery, symptoms indistinct

in a suggested respective order of sensitivity to one or the other virus: sesame, lima bean, snapdragon, spinach, watermelon, eggplant, *Phacelia campanularia*, *Gomphrena globosa*, petunia, *Chenopodium amaranticolor*, and *Gypsophila elegans*.

DISCUSSION

Price (18) first described TomRSV as TRSV No. 2 to distinguish it from TRSV described by Wingard (23). TRSV No. 2 was later referred to as TomRSV by Holmes (11). This nomenclature assumed that the virus was predominant on tomato. The only report of a natural occurrence of TomRSV on tomato is in Indiana by Samson and Imle (20), but the restricted host range indicates that this virus was probably different from Price's (18). Several workers (1, 13, 21) failed to transmit TomRSV from different hosts to tomato. Oregon culture TomRSV No. 644 was isolated from a naturally infected cucumber[2]. The virus was identified recently from bulbous Iris (9); it is seed-borne in soybean (6) and is reported from various hosts (2, 4, 10, 13, 17, 21). Karle (13) showed a similar host range for the peach yellow-bud-mosaic virus to that reported for TomRSV from florists' hydrangea (4). Cadman and Lister (6) showed a serological relationship of peach yellow-bud-mosaic virus to TomRSV. Karle (13) reported cotton as an experimental host of peach yellow-bud-mosaic virus but was unable to infect watermelon. That cotton (Fig. 1) was infected and watermelon was not infected by the TomRSV used in the present studies might add support to the relationship of these viruses. Texas Agricultural Experiment Station workers have observed symptoms in the field on tomatoes and cotton similar to those produced experimentally by TomRSV[3].

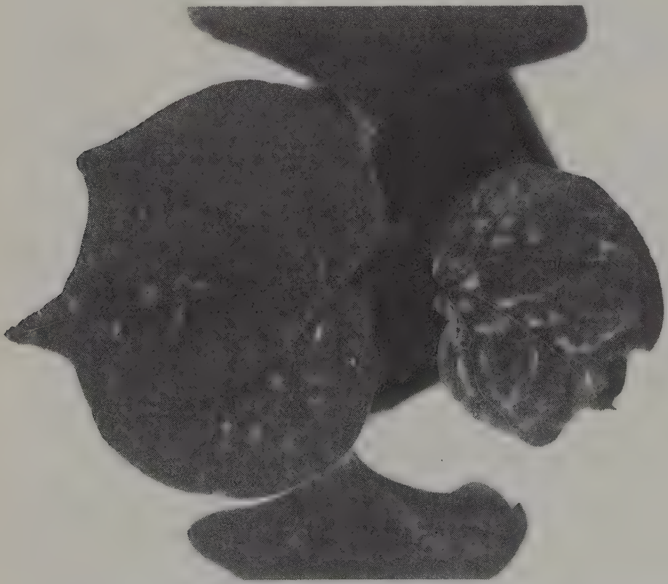

FIGURE 1. Young cotton plant inoculated with TomRSV. Note systemic chlorotic spots, interveinal chlorosis, and leaf distortion.

Reactions of experimental test species to repeated TomRSV inoculations were not always identical; apparently, virus titre varied in the source plants. There was no correlation between symptoms expressed on test species and intensity of symptoms on the virus source plants. It was nearly impossible to detect symptoms of TomRSV in certain test species in a glasshouse at temperatures above 85° F. Symptoms of TRSV were readily expressed at temperatures up to 95° but were frequently masked in older plants.

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SYMPTOMS OF TOBACCO RINGSPOT VIRUS ON PAPAYA

D. M. McLean and E. O. Olson[1]

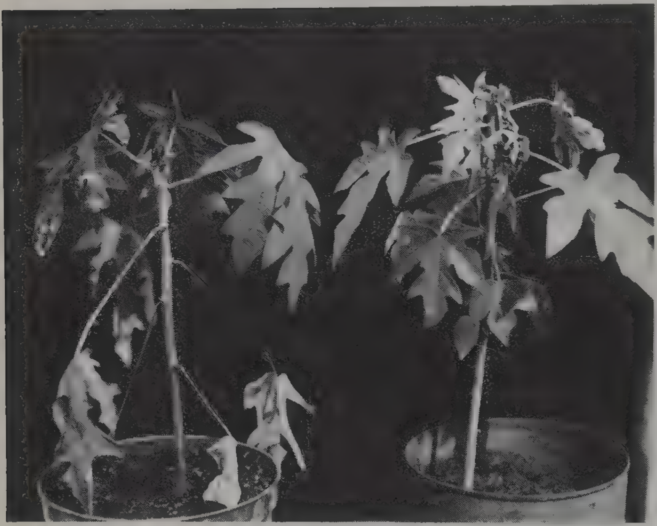

FIGURE 1. Inoculated papaya plants showing symptoms of tobacco ringspot virus infections. Left -- Plant showing terminal necrosis and collapsed, wilted leaves. Right -- Plant showing collapse of terminal leaves, an early symptom of infection.

During routine inoculations with tobacco ringspot virus in a study of common weed hosts as reservoirs of the virus infection on vegetables at Weslaco, Texas (2), papaya (Carica papaya) was very sensitive when mechanically inoculated with the virus from triturated cucumber leaves. The virus was readily transferred from papaya back to cucumber by the same procedure. Inoculations were made during July and August 1962 in a shaded glasshouse, where diurnal temperatures ranged from 75° to 100° F, and also out-of-doors in September.

Six to 8 days after inoculation of papaya seedlings 4 to 6 inches tall, the leaves wilted and drooped; some plants showed terminal necrosis. Wilted plants did not recover when they were removed from soil and their roots suspended in distilled water. All inoculated plants died. Plants that were older (10 to 14 inches to 3 feet) when inoculated suddenly collapsed or showed yellowed, crinkled leaves which drooped on flaccid petioles and soon abscised (Fig. 1). Faint chlorotic spots appeared on some terminal leaves; roots looked healthy and vascular discoloration was absent. Symptoms of tobacco ringspot infections resemble those described on diseased papaya in Hawaii by Parris (4) or those of "yellow crinkle" reported by Simmonds (5) in Australia.

It is sometimes difficult to grow mature, fruiting papaya plants in the Rio Grande Valley and the decline or collapse of plants is not uncommon (3). Decline in older plants previously has been attributed to root rots caused by soil-borne organisms or to salinity (1). Tests for tobacco ringspot virus have not been made on declining, fruiting papaya in the Rio Grande Valley. Since the virus is ubiquitous in soils in this region it is suspected as a cause of papaya decline.

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GUMMY STEM BLIGHT RESISTANCE OF INTRODUCED WATERMELONS[1]

Grover Sowell, Jr. and Gerald R. Pointer[2]

Abstract

P.I. 189225[3] of *Citrullus vulgaris* consistently demonstrated resistance to *Mycosphaerella citrullina*.

INTRODUCTION

Gummy stem blight, caused by *Mycosphaerella citrullina* (C. O. Sm.) Gross., is the most destructive disease of watermelon, *Citrullus vulgaris*, in Florida according to Schenck (4). Wall and Grimball (8) stated that the disease is of sufficient severity in the southern United States to warrant the attention of plant breeders. According to a report from Australia (1), the disease caused losses ranging from 30 to 80% of the crops in one area during the 1960-61 season. There have been no published reports of resistance in watermelon.

MATERIALS AND METHODS

Single rows of the 439 introductions of *Citrullus vulgaris* maintained by the Southern Regional Plant Introduction Station were tested for resistance to *M. citrullina* in a series of preliminary screening tests conducted in the greenhouse. A single-colony isolate of the pathogen (CS-1), which was used for all inoculations, was obtained from infected cantaloup plants collected at the United States Vegetable Breeding Laboratory, Charleston, South Carolina. Isolates obtained from other research workers were not superior to CS-1 in the consistent production of highly pathogenic inoculum. The technique used in producing inoculum is a modification of that reported by Barnes and Epps (2). The pathogen was grown on autoclaved frozen snap beans in 125-ml Erlenmeyer flasks for 7 to 10 days under fluorescent light. Cultures were held at room temperature (25° ± 5° C). Two cultures were blended in 500 ml of distilled water with a food blender for 2 minutes. This procedure was repeated for each liter of inoculum to be prepared. Filtration of inoculum through Whatman No. 4 filter paper removed most of the snap bean substrate but allowed the conidia to pass through. No attempt was made to adjust the spore concentration of the inoculum. Spore counts made in all except the preliminary tests and the first replicated greenhouse test indicated that the inoculum contained from 1 to 5 x 10^5 spores per ml.

A technique similar to that used by Sowell (7) was employed in growing the plants and in applying the inoculum. Lime was added to a mixture of three-fourths soil and one-fourth peat moss at a rate equivalent to a field application of approximately 2 tons of dolomitic limestone per acre furrow slice. A 20-20-20 soluble fertilizer was applied at the rate of approximately 10 grams per liter of water once a week after the plants emerged. After the initial spraying of each row with the inoculum, the plants were sprayed again by moving the nozzle back and forth in a direction perpendicular to the direction of the first spraying until some run-off occurred. After inoculation, the plants were incubated for 48 hours at 25° ± 2° C in a saturated atmosphere. Data on the total number of plants, the number of plants dead, and the infection index were recorded 1 to 2 weeks after inoculation. The infection index is a modification of the disease-rating system agreed upon by the plant pathologists of the four regional plant introduction stations at their 1960 meeting at Beltsville, Maryland, and consists of the following scale: 0 = no infection, 0.5 = trace to 10%, 1.0 = 11-20%, 1.5 = 21-30%, 2.0 = 31-40%, 2.5 = 41-50%, 3.0 = 51-60%, 3.5 = 61-70%, 4.0 = 71-80%, 4.5 = 81-90%, 5.0 = 91-100%. This index was used to evaluate the percentage of total plant area affected by the disease.

Introductions with an infection index of 2.0 or less and 30% or less of the plants dead in preliminary screening tests were selected for further testing in replicated tests. One exception was made by including in the replicated tests an introduction with an index of 3.5 but a very low percentage of plants dead. Spalding and Charleston Gray were included as standard susceptible varieties. The design of this test was a randomized block with four replications. The disease-index data were analyzed as such but the percentages of plants dead were converted to angles as recommended by Snedecor and Cochran (5) before the analysis of variance was calculated. Duncan's multiple range test (3) was used to compare the means.

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[3] Plant Introduction number.

In the summer of 1961 the five plant introductions with the lowest infection indices in greenhouse tests were compared with Charleston Gray in the field. In this experiment the plants were grown in peat pots in the greenhouse, inoculated, and evaluated for resistance to the disease before transplanting. The surviving plants were transplanted to the field. Plots which contained an inadequate number of surviving plants were replanted with seed 1 week later. On several days during the test the plants were inoculated at dusk with a suspension of conidia prepared from snap bean cultures of the pathogen. In a second replicated greenhouse test four of the five lines grown in the field were compared for resistance. Charleston Gray was included as a susceptible standard. P. I. 171392 was omitted since the seed of this introduction did not germinate. In a third greenhouse test P. I. 189225 and P. I. 186975 were compared with Charleston Gray and Blacklee.

RESULTS

Sixteen introductions of watermelon were resistant in the preliminary screening tests. In the first replicated greenhouse test P. I. 189225 was the only introduction with an infection index significantly lower than the standard susceptible varieties (Table 1).

Table 1. Severity of gummy stem blight on watermelon seedlings in three replicated greenhouse tests.

Test number	P. I. number or variety	Infection index		Percent plants dead	
1	189225	2.6	a**	38.1	a
	189317	3.0	ab	43.8	ab
	171392	3.1	ab	39.6	ab
	186975	3.2	ab	41.0	ab
	255136	3.3	ab	49.3	ab
	Spalding	4.7	b	82.6	b
	Charleston Gray	4.7	b	73.0	ab
2	189225	1.1	a	14.8	a
	186975	3.4	b	53.2	b
	189317	3.5	b	53.0	b
	255136	3.5	b	53.6	b
	Charleston Gray	4.9	b	86.2	b
3	189225	0.9	a	7.9	a
	Charleston Gray	4.4	b	78.3	b
	186975	4.4	b	77.5	b
	Blacklee	5.0	b	98.9	b

**Means not followed by a letter in common are significantly different at the 1 percent level according to Duncan's multiple range test.

Table 2. Severity of gummy stem blight on watermelon seedlings in the 1961 field test.

P. I. number or variety	Infection index		Percent plants dead	
171392	0.3	a**	0.0	a
189225	0.3	a	9.5	ab
186975	0.7	a	15.8	ab
255136	2.2	ab	36.8	bc
189317	2.7	b	50.0	bc
Charleston Gray	4.0	c	79.0	c

**Means not followed by a letter in common are significantly different at the 1 percent level according to Duncan's multiple range test.

This introduction was not statistically superior to Charleston Gray when resistance was evaluated by the percentage of plants dead.

In the second and third greenhouse tests, P.I. 189225 was significantly superior to the other introductions and to the susceptible standard varieties when resistance was evaluated by both measures of resistance. The growth of this introduction also was superior.

In the field test the infection indices of P.I. 171392, P.I. 189225, and P.I. 186975 were significantly lower than those of P.I. 189317 and Charleston Gray before transplanting (Table 2). The disease was not severe enough on the surviving plants, after transplanting to the field, to allow a further evaluation of their resistance. The size of the fruits agreed with those previously reported (6), except that the fruits of P.I. 189317 were 7.5 to 8 inches in diameter. The fruits of P.I. 189225 showed dark green stripes on a background of lighter green. The interior of the fruits of this introduction was pale green, and the flesh had a cucumber-like flavor. The fruits averaged 5 inches in diameter.

P.I. 189225 appears to be sufficiently resistant to the pathogen to justify its use by plant breeders as a source of resistance. Further research on the relative resistance of P.I. 189225 and other resistant introductions is needed.

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EFFECT OF LIMING OF SOIL ON BARLEY POWDERY MILDEW

J. Vlamis and C. E. Yarwood

Summary

In greenhouse trials, the addition of lime to a moderately acid soil of the Wilder series increased the growth of barley, increased the growth of powdery mildew on this barley, and increased the damage to barley caused by this infection.

INTRODUCTION

In general, a vigorous host favors infection with powdery mildews (Erysiphaceae), but many treatments which increase or decrease plant growth do not necessarily increase or decrease susceptibility (9). In reports that high pH of soil favors the development of mildew on foliage (2, 6), plant growth and powdery mildew development were not given quantitatively, and it cannot be safely decided if the increased mildew development was because of, or in spite of, the effect of the treatment on the host. In the present study a quantitative relation of liming to plant growth and mildew susceptibility is reported.

PROCEDURE

Barley plants (*Hordeum vulgare* var. Atlas 46) were grown, nine plants per pot, in 6-inch pots containing 1600 grams of soil of the Wilder series. This soil is derived from sandstone under about 50 inches of rainfall and has a pH of about 4.8. It was found to be infertile in greenhouse pot tests and this was rectified by the addition of nitrogen, phosphorus, and sulfur to all pots. One half of the pots were each given 6.4 grams of calcium carbonate, which is equivalent to 4 tons of lime per acre. This raised the reaction to about 5.7 and was sufficient to eliminate the deleterious effects of the high acidity on the growth of barley.

The plants in half of the 4 to 16 pots in each trial were inoculated at about 2 weeks from seeding with mildew (*Erysiphe graminis* DC.) by dusting them with conidia from other infected plants. In the trials reported here, inoculation was deliberately light because preliminary work showed that the effect of soil pH on mildew was not clearly apparent when infection was so heavy that the lesions on the limed plants were contiguous. Mildew was recorded at about 2 weeks after inoculation, and plants were harvested about 2 weeks later.

Results are presented in Table 1. Plants in limed soil had about three times as many mildew lesions as plants in unlimed soil. The average reduction in green weight due to mildew was 61% for plants in limed soil and 19% for plants in unlimed soil. Liming caused a 59% increase in the green weight of healthy plants. The apparent 23% reduction in green weight due to liming of the mildewed plants is likely because the reduction in green weight of these plants due to mildew was greater than the expected increase due to lime in the absence of mildew.

Table 1. Effect of liming of soil on barley powdery mildew.

	Unlimed	Limed	Limed / Unlimed
pH	4.8	5.7	
Mildew lesions per plant (5 trials)	22.5	70	3.1
Green weight of barley per pot, control	8.8	14.0	1.6
Green weight of barley per pot, mildewed	7.1	5.5	.77
Dry weight[a] of barley per pot, control	2.6	3.7	1.4
Dry weight of barley per pot, mildewed	2.5	2.6	1.0

[a] After 24 hours at 70°C.

DISCUSSION

The addition of lime to the acid soil used in these experiments improved it as a medium for the growth of barley. It also made barley a more suitable host for powdery mildew. The factors responsible for the improved growth of certain higher plants have been worked out, more or less (4, 5, 8). Acid soils have been found to be low in bases such as Ca, Mg, and K, and high in hydrogen, aluminum, and manganese. One or more of these factors may be involved in the development of mildew on plants grown in acid soil with or without lime.

The possibility that hydrogen ions are involved directly is not likely in view of work which has shown that plants grown over a wide range of pH maintain a remarkably stable pH of the cell sap (1). A more likely prospect is a high manganese content having toxic effects on barley and rendering it a less suitable host for mildew. Plants grown in acid soils are noticeably higher in manganese than plants grown in soils of a higher pH (5, 7). Sand culture work also showed that high Mn in the growth medium had an adverse effect on powdery mildew on wheat (3). The higher succulence of barley grown in limed soils may also be a contributing factor. Still left uncertain are the possible roles of Ca, Mg, and K which are deficient in acid soils, and aluminum which is present in toxic amounts.

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ASPERGILLUS CROWN ROT OF PEANUT IN GEORGIA[1]

Curtis R. Jackson[2]

Summary

Aspergillus niger, the reported cause of a peanut seedling disease in Africa, Asia, and Australia, was found to be the cause of a crown rot of peanuts in Georgia. The disease commonly results from invasion of the hypocotyl directly or by way of the cotyledons in absence of injured tissue. Radicle infection occurs occasionally. Infected seeds and soil-borne mycelia were noted as important sources of inocula for field infection.

Aspergillus crown rot was first reported in 1926 by Jochems (9) who offered evidence that peanuts were subject to a stem rot caused by Aspergillus niger Van Tieghem. Morwood (12, 13) later reported the disease from Australia and gave additional proof of the pathogenicity of A. niger. Gibson (5, 6) reported the disease from East Africa and established valuable etiological information. In India, Jain and Nema (8) found A. niger attacking peanut seedlings and Nema, et al. (14) later reported on further studies of the disease.

In the United States, A. niger has not been reported as the cause of a peanut seedling disease. Garren and Wilson (4) gave results of isolations made in Georgia in which Aspergillus spp. were rather frequently found in plants with "collar rot." Garren and Higgins (3) found Aspergillus spp. in sound and visibly damaged peanut kernels but not in seed with concealed damage. However, Heiberg and Ramsey (7) noted that Aspergillus spp. were present in seed with concealed damage. Diener (2) listed A. niger as frequent among the Aspergilli he found in stored peanuts.

In 1951 Garren and Wilson (4), summarizing extant information about peanut diseases, discussed the possible causes of "collar rot," a stem-rotting disease of established seedlings. They also noted the similarity between Aspergillus crown rot and the "collar rot" disease prevalent in the southeastern United States. McGuire (11) showed that "collar rot," at least in the area of his studies, was due to Diplodia gossypina Cke. His results indicate that the development of Diplodia collar rot depends on the predisposing effects of incident and reflected heat, near the soil line. The resulting heat-injured tissue is quickly invaded by D. gossypina. Boyle (1) established a valid distinction between heat canker and "collar rot"; heat injuries may be lethal per se or may be invaded by D. gossypina or other fungi.

DISEASE INCIDENCE IN GEORGIA

Although Aspergillus crown rot apparently had not occurred frequently prior to 1961, conversations with research and extension workers reveal that the disease was noted during the past decade. During May and June 1961, several specimens of dying seedlings, having characteristic symptoms and signs of Aspergillus crown rot, were received from farmers in Georgia. Cultures of hypocotyl and epicotyl tissues adjacent to necrotic areas yielded A. niger in all cases. Field observations and county agents' reports in 1961 indicated that stand losses were great in 15 of the 84 peanut-growing counties of the State. These reports and observations did not distinguish between insect and disease losses. However, in one instance where insects were not present an estimated stand loss of 40% was due mainly to Aspergillus crown rot.

In 1962 seedling death reached greater proportions, and A. niger was cultured from 71% of 150 plants submitted for diagnosis. These plants comprised 10 samples collected in the field by county agents and farmers and presumably were typical of each disease situation. Rhizopus sp., Fusarium spp., Aspergillus spp., and Diplodia sp. were cultured from the remaining plants. Detailed field observations were obtained in 1962. Extremely dry weather prevailed when most peanut plants were in the seedling stage. Damage due to the lesser corn stalk borer, Elasmopalpis lignosellus (Zeller), was widespread and the southern corn root worm, Diabrotica undecimpunctata howardi Barber, was destructive in a small area of the State. Extensive seedling death was reported from 24 counties and in many cases was verified as due to Aspergillus crown rot. No clear association was evident between insect injury and crown rot since both conditions were found together in some fields or singly in other fields. In three separate fields, where stands were reduced by 30 to 50%, all dying plants that were examined by the writer had Aspergillus crown rot. Stand reductions of 10 to 30% were common. Peanut varieties commercially classified as "Runner types" (10) were found to be affected with the disease more frequently than "Spanish types."

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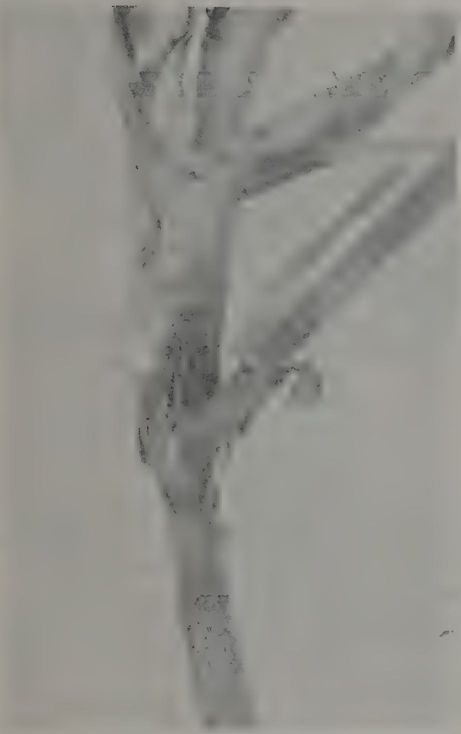

FIGURE 1. Cotyledonary portion of peanut seedling shows advanced symptoms of shredding and discoloration typical of Aspergillus crown rot.

SYMPTOMS AND SIGNS

Aspergillus crown rot is characterized in the field by sudden wilting of the entire plant, or of one or more branches, and subsequent death of the plant accompanied by rotting of the tissue of the cotyledonary node and the adjacent hypocotyl. The infected tissue is at first brown, becoming lighter in color with age. Under dry conditions the necrotic tissue becomes distinctly shredded in appearance (Fig. 1). Necrosis and shredding of tissue may extend several centimeters up the branches of the epicotyl. Infection of the radicle or the hypocotyl, at a point below the cotyledonary node, occurs rarely in the field. Under experimental conditions of abundant soil inoculum, radicle or basal-hypocotyl infection may occur frequently. The most commonly observed initial sites of natural infection are the cotyledons. Clusters of conidiophores and black spore masses can be seen readily on the infected plant parts above and below the soil surface. Development of conidia usually occurs by the time wilting is evident. Morwood (13), Nema, et al. (14), and Gibson (5) have reported similar symptoms and signs.

CAUSAL ORGANISMS

Most isolates from rotted crown tissue and from infected seed conform to the description of A. niger given by Thom and Raper (17). A single pathogenic isolate from an infected seed in 1962 is more closely allied to Aspergillus awamori Nakazawa on the basis of sterigmata length. The fungus originally reported by Jochems (9) to be A. niger was later identified as Aspergillus pulverulentus (McAlpine) Thom. Other reports of the peanut crown rot organism give only A. niger. Thus, within the A. niger group as presently established (17), isolates of several species, differing slightly in morphology, may exhibit similar pathogenic characteristics.

PATHOGENICITY OF ASPERGILLUS NIGER

Since the ubiquitous occurrence of A. niger and its predominantly saprophytic mode of existence impugn its possible primary pathogenic nature, the pathogenicity of isolates derived from necrotic peanut crown tissue and from naturally infected seeds was tested.

Methods: Thirty-six 4-inch clay pots were each two-thirds filled with white quartz sand and autoclaved for 1.5 hours at 16 psi. Three hand-shelled seeds of Early Runner variety were planted in each pot. Previous analysis of seeds from the same lot indicated that 6.6% of the seeds contained internal inoculum of A. niger. When the seedlings were approximately 6-cm tall the cotyledons and bases of branches were covered by adding a 3-cm layer of infested sand or soil, or sterile sand, over the surface of the existing sand. These materials were added carefully to avoid wounding the plants. The additives were prepared by mixing pure

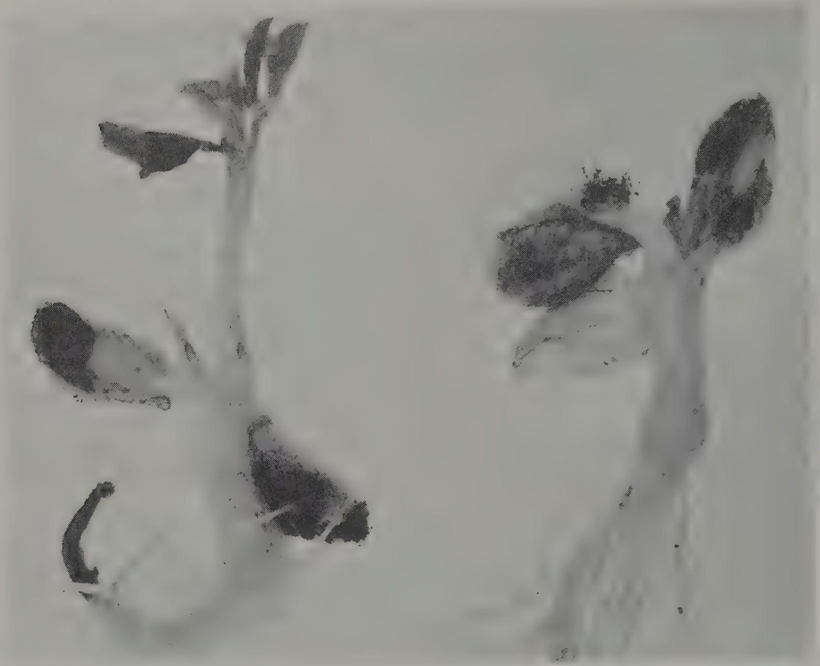

FIGURE 2. Naturally infected seeds germinated in sterile sand resulted in these seedlings. Plant on right with broken epicotyl shows basal cotyledon infections. Plant on left has infected cotyledons and radicle. Radicle infection occurred during germination when cotyledons and radicle were in contact with each other.

cultures of the fungus with autoclaved sand or screened sandy, loam soil. Pure cultures of pooled isolates of the fungus were obtained by infesting previously dried and propylene oxide-fumigated peanut branches; the semi-dry cultures were fragmented in a Waring Blendor before mixing. Infested sand, soil, or sterile sand were each added to a series of 12 pots. Potted plants were placed in saucers on a bench in a heavily whitewashed greenhouse. Ambient air temperatures during incubation did not exceed 41° C.

Results: Seedling death due to Aspergillus crown rot was evident 15 days after inoculation and plants were removed as they died. Plants in pots to which sterile sand was added also developed the disease. The position of most lesions indicated that hypocotyl invasion was the result of, or was associated with, cotyledon infection. When the experiment was terminated 35 days after inoculation, the percentages of plants killed by A. niger were: infested sand - 64%, infested soil - 46%, and sterile sand - 11%. All dying plants were cultured on potato-dextrose agar (PDA) and in every case A. niger was recovered from marginal lesion tissue.

Discussion: Several methods have been employed in studies of the pathogenicity of A. niger to peanut. Morwood (13) and Nema, et al. (14) used injured and uninjured seed which had been inoculated with spores or fragments of cultures, or wounded the hypocotyl and exposed it to infested soil. Gibson (5) found that mycelial inocula were essential to infection of growing plants.

In the present investigation, inoculation with mycelial inocula in soil placed in the area of the cotyledons and hypocotyl resulted in considerable infection in absence of intentional wounds. Possible heat and insect injury were excluded by precautions used in the experiment.

The small percentage of plants infected in the pots receiving sterile sand was due to use of seed infected with A. niger. Infected seed have been used to reproduce the disease as cited above. Subsequent experiments with naturally infected seeds have shown that seed lesions commonly enlarge, as cotyledons expand, and lead to rotting of the cotyledons. Invasion of hypocotyl tissue from infected cotyledons usually, but not invariably, follows (Fig. 2). The fungus in infected cotyledons, if in contact with the hypocotyl or radicle during germination, rapidly invades these tissues causing pre-emergence death. These observations tend to confirm field observations reported by Gibson (5).

Gibson's (5) observation that the development of crown rot was most severe in soils of light texture may help to explain the greater incidence of crown rot resulting from use of infested sand.

This evidence, showing that infection by A. niger can occur in absence of wounds, does not preclude the possible and perhaps frequent invasion by this fungus of insect wounds, heat injuries, cultivation injuries, and necrotic tissue resulting from infection by other pathogens. Nema, et al. (14) have shown that deliberate hypocotyl wounding results in a greater incidence of crown rot than occurs in unwounded plants. The extent to which saprophytic colonization of injured or infected tissue occurs is not known.

NATURAL SOURCES OF INOCULA

Two instances of 40 to 50% stand loss due to Aspergillus crown rot, which occurred in different counties, led to investigations to establish the principal sources of field inocula. In both disease situations Early Runner variety had been planted and the disease became severe at 20 to 30 days after planting.

Methods: A sample of the seed lot used for each planting was obtained from each grower and composite soil samples were collected from the upper 6 cm between rows in each field. Soil samples were air dried and passed through a 1-mm screen. The soil dilution plate technique devised by Schenck and Curl (16) was used with Rose Bengal-streptomycin medium. Six replications were prepared from each soil sample and were incubated at 28° to 32° C for 5 days. Replications were averaged to obtain the estimated population.

Sixty-five seeds from each lot were randomly chosen and placed in separate sterile 500-ml flasks. Two hundred ml of 200 ppm Hyamine 3500 (50% n-alkyl (C_{14}, C_{12}, C_{16}) dimethyl benzyl ammonium chlorides) were added and each flask was shaken on a Burrell wrist-action shaker for 2 minutes. After this solution had been decanted and replaced with 200 ml of 0.52% NaOCl, the flasks were shaken again for 2 minutes. The seeds were aseptically placed in separate test tubes containing sterile PDA and kept at 28° to 32° C until the germinating seedlings were approximately 6 cm tall.

Results and Discussion: A comparison of the seed and soil analyses from the two fields is given in Table 1.

Table 1. Estimated numbers of A. niger propagules in soil and percentages of infected seed.

	A. niger propagules per g of soil	% seeds with A. niger infection	Approximate % plant loss
Field A	7620	0.8	50
Field B	165	43.0	40

A. niger was the only organism that grew in dilution plates prepared from the soil from field A. The soil from field B yielded a moderately large number of Rhizopus nigricans Ehrb. in addition to A. niger. The seeds planted in field A yielded no other fungus but seeds planted in field B also yielded Aspergillus spp., R. nigricans, Trichoderma viride Pers. ex Fr. and a Penicillium sp.

Soil samples from other fields where Aspergillus crown rot had occurred in abundance were examined by the same methods. Estimated abundance of A. niger was commonly 1000 to 10,000 per g soil and reached 125,000 per g. Unfortunately, seeds were not available from these plantings for comparisons like that given in Table 1.

Two sources of natural inocula, soil- and seed-borne, are evident from these results. The relative importance of the seed-borne source is now being elucidated in a survey of the amount of A. niger infection in representative peanut seed lots. Thus far results indicate that peanut seed lots with more than 10% A. niger infection are not common.

CONTROL

Field control studies for this post-emergence disease in Australia and East Africa have involved the effects of seed treatments on the incidence of the disease. Gibson (6) first reported that organo-mercurial seed treatments led to an increase in crown rot. He demonstrated that certain isolates of the fungus were mercury tolerant and hypothesized that the partial soil sterilization produced by organo-mercurial materials operates to the advantage of A. niger mycelium. Morwood (13) reported that there appeared to be less post-emergence loss of plants after seed treatment with thiram than after seed treatment with Ceresan or Agrosan. Nema, et al. (14) tested several organo-mercurial seed treatments and found that, compared with the untreated control, all materials reduced pre- and post-emergence death of peanuts. In a recent report on field control of pre-emergence rot and Aspergillus crown rot of peanuts, Purss (15) reported results of experiments in Australia with various seed-treatment materials. Although mercury compounds were effective in preventing pre-emergence rotting, they were associated with a significantly higher incidence of crown rot than occurred in the untreated control or with materials such as captan and thiram. Purss concluded that the use of straight organo-mercurial compounds on peanut seed should be avoided because of the increase in crown rot often associated with this practice.

Research now in progress in Georgia is concerned with evaluation of seed treatments as they affect pre-emergence seedling diseases and Aspergillus crown rot.

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OCCURRENCE OF TWO BACTERIAL DISEASES ON NEW HOSTS IN ISRAEL[1]

Zafrira Volcani

A bacterial soft rot disease of collar and stem on fodder-cabbage occurred in Israel for the first time during late autumn of 1960 in different locations (two in the Coastal Plain and one in the Beit Shean Valley). The incidence of the disease, however, was moderately low. Similar bacterial organisms were isolated in pure culture from infected parts of plants in all three localities, and were found to be the cause of the disease. Under conditions of relatively high humidity, the organisms and their re-isolates produced softening on stems of fodder-cabbage, as well as on cabbage and cauliflower, when inoculated on young plants through wounds. Examination of the organisms revealed that they were identical to Pectobacterium carotovorum var. aroideae Dowson (2, 3) or Erwinia carotovora (Jones) Holland (1) var. aroideae Dowson (2, 3), which was previously found to cause soft rot disease on several other hosts in Israel (5, 6, 7, 8, 9, 10). Like other organisms of the same group they are wound parasites, inducing softening of fruits and vegetables under relatively high humidity. They caused black leg symptoms and softening of stems when inoculated on potato and tomato plants, respectively. The optimum temperature for infection was 28° to 30° C, with a minimum of 8° and a maximum of 39° to 40°

Melon fruits of the Spanish Honey Dew variety were affected with partially soft or hard necrotic brown large spots in a field at Carmia (southern part of the country) in June 1961[2]. The lesions were caused by a spore-forming bacterium identical to Bacillus polymyxa (Prazmowski) Migula (1), which was previously found to be the causal agent of a spot disease on sweet pepper fruits in Israel (4, 11). The same organism was also isolated later from rotten potato tubers in the field[3]. In inoculation tests through wounds, the melon organism and its re-isolates produced partially soft brown spots on melon fruits. Under conditions of relatively high humidity it induced partially soft and soft spots on green pepper and tomato fruits, as well as softening of potato tubers at temperatures of 30° to 38° C.

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[1] Contribution from the National and University Institute of Agriculture, Rehovot. 1962 Series, No. 532-E.

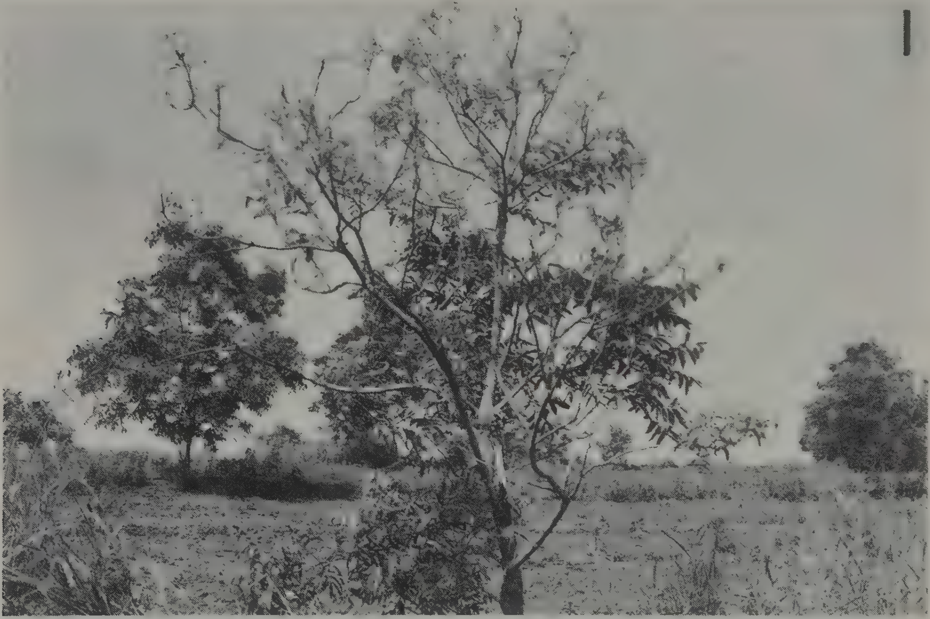
[2] Samples were submitted by Mrs. N. Temkin-Gorodeski of the Division of Storage Research of Fruits and Vegetables.

[3] Volcani, Zafrira. 1951. Unpublished data.

UNUSUAL CASE OF DELAYED FOLIATION AND DIEBACK OF MONEYMAKER PECAN

J. R. Cole and J. H. Hunter[1]

In May 1953, some abnormal pecan trees of the Moneymaker variety, in a planting mixed with Moore trees, were called to the attention of the writers by William and Clyde Wilson of Fort Valley, Georgia. Twelve Moneymaker trees in a planting of approximately 80 nine-year-old trees were smaller than the others of both varieties, showed evidence of terminal dieback, and had poorly developed leaves on some of the live terminals, whereas the sprouts on the main trunks were making unusually vigorous growth (Fig. 1). These sprouts had abnormally large leaves, ragged along the margins and cupped slightly upward. The shoots bearing poorly developed leaves grew, but the leaves developed little. In some instances pistillate flowers formed in the terminals set nuts before foliage developed. Foliage which formed several weeks later came from buds in the axils of the affected leaves. Occasionally, some of the buds formed catkins in late May or early June (Fig. 2). Seemingly the dieback associated with the malady is due to winter injury to the shoots, which were not so physiologically mature as normal shoots.



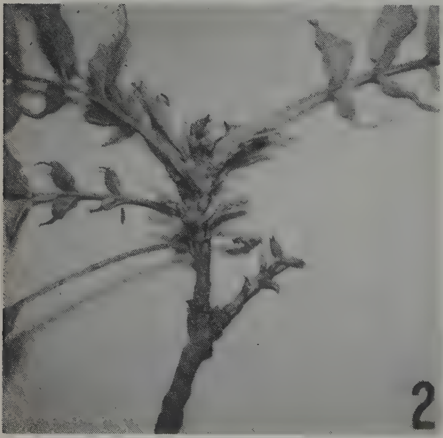


FIGURE 1. Type of growth on affected Moneymaker pecan trees. (Photo June 4, 1957)

FIGURE 2. Branch from affected tree showing nuts in normal position of present year's growth with leaves and staminate flowers developing from buds which would normally stay dormant until next season. (Photo May 29, 1961)

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[1] Plant Pathologist and Soil Scientist, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

No Moore trees were affected and the unaffected Moneymaker trees appeared normal for their age and were bearing crops of nuts (normal trees shown in background of Figure 1). The owners of the orchard had thought that the trees were affected with rosette, a zinc-deficiency, and applied zinc sulfate prior to 1953 without observing any beneficial results.

This abnormal condition has also been observed in orchards near Adel, Boston, and Dawson, Georgia. All the affected trees have one characteristic in common: they are 10 to 20 years old. The disorder apparently has not spread to other Moneymaker trees or varieties planted in the same orchard.

Scions and grafts from affected trees grew normally when transferred to normal trees and the normal trees were not affected. Various fungi and bacteria isolated from affected trees failed to produce the disorder when transferred to healthy trees and were apparently saprophytes. Inoculations of healthy trees using crushed leaves and juices from affected trees failed to reproduce the abnormal condition.

Leaves from the affected trees were abnormally high in zinc and the soil from around the trees was highly acid (pH 4.7 to 4.9). Applications of lime corrected the soil acidity and reduced the content of zinc in the leaves to less than half that found in the original samples. Improvement in the growth of the trees resulted from the application of lime, but it failed to correct the delayed foliation permanently; the response to the lime was apparently due to lowering the content of zinc in the trees.

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ALBANY, GEORGIA

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INDEX TO VOLUME 46

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Crops Research Division
Volume 46

Plant Industry Station, Beltsville, Maryland
Index Number

AUTHOR INDEX

ADAM, A. V., 366
ADAMS, ROBERT E., (281)
ALBERT, J. J., 163
ALCORN, STANLEY M., 156
ALLEN, ROSS M., (667)
ANCALMO, OSCAR, 293
ANDERSON, J. C., (277)
ANZALONE, LOUIS, Jr., 213
ASTIN, J. S., Jr., 738
AUSTENSON, H. M., (256), (579)
AVIZOHAR-HERSHENZON, ZEHARA, 418

BACHE, C. A., (844)
BACHELDER, STEPHEN, 320
BAIN, DOUGLAS C., 55
BAKER, RALPH, (506)
BARKSDALE, T. H., (404)
BARNES, GEORGE L., 192, 236
BASAK, WLADYSLAW, 404
BAXTER, JOHN W., 706
BEACH, ROBERT E., (64)
BECK, E. W., (240)
BEGA, ROBERT V., 107, (269)
BELKENGREN, R. O., 119
BENSON, N. R., (59)
BERAHA, LOUIS, 354
BERGESON, G. B., 535, (623)
BERNARD, R. L., (181)
BERRY, F. H., 521
BHATT, DIBYA DEO, 342
BIERLEIN, DELBERT L., 838
BIRCHFIELD, WRAY, 683
BIRD, G. W., (858)
BITTERS, W. P., (267)
BLANK, L. M., 396
BORDERS, HUEY I., 651, 652
BOSHER, J. E., 92
BOWER, CLYDE A., (463)
BOYCE, JOHN S. Jr., 63
BRASE, K. D., (356)
BRENNAN, EILEEN, (140), 792
BRIERLEY, PHILIP, 335, 505, 625, 627
BRISTER, L. R., (683)
BRITTON, M. P., (9), (724)
BROWN, R. T., (185)
BROYLES, JAMES W., 8
BRUEHL, G. W., 579
BURNETT, HARRY C., 279, 348, 692, 693
BURTS, E. C., (59)
BYRD, JOHNNIE, (677)
BYRD, W. P., (438)

CADMAN, C. H., 770
CALDECOTT, R. S., (88)
CALPOUZOS, L., 105, 758
CAMPANA, RICHARD, 265
CAMPBELL, FRED G., (305)
CANNON, ORSON S., (176)
CAPPELLINI, R. A., 31, 246, (290)
CARPENTER, T. R., 750
CARVER, R. G., (591), (762)
CETAS, R. C., 601
CHAMBERLAIN, D. W., 707
CHAMBERS, ALBERT Y., (48)
CHANDLER, W. A., 476, 831
CHAPMAN, R. A., (372)
CHEN, TSEH-AN, 346, 839
CHENEY, P. W., (722)
CHO, YONG SUP, (777)
CHORIN, M., 790
CHRISTENSEN, C. M., (785)
COBB, F. W., Jr., (194)
COBB, GRACE S., 541
COCHRAN, J. H., 617
COCHRAN, L. C., (495)
COFFMAN, F. A., 438
COHN, E., (531)
COLBERG, CARLOS, (105), (758)
COLE, J. R., 894
COOK, A. A., 569
COOPER, W. E., (462), (609), 640
COURTNEY, WILBUR D., 256
CRAIG, DONALD L., (586)
CRANDALL, P. C., (579)
CRISPIN, ALFONSO, 411
CROSIER, W. F., 111
CROSSAN, D. F., 227
CUNNINGHAM, H. S., (631)

DAINES, ROBERT H., (140), (246), 251, 253, 513, (792), 808
DAMSTEEGT, V. D., (579)
DARBY, J. F., 441
DAVEY, C. B., (646)
DAVIS, J. R., (688)
DAVIS, SPENCER H., Jr., (393)
DAVIS, T. S., (261)
DAVIS, WILLIAM C., (293)
DAVISON, ARLEN D., 310
DESAI, M. K., (381), (874)
DESAI, M. V., (173)
DESJARDINS, PAUL R., 414
DeWOLFE, T. A., (606)
deZEEUW, D. J., (93)
DICKENS, LESTER E., (274)
DICKEY, ROBERT S., 73
DIENER, URBAN L., 232
DIETER, C. E., (441)
DONGO, SEGUNDO, (411)
DOOLING, O. J., (521)
DRAKE, CHARLES R., 509
DRAKE, R. J., (211)
DRIVER, CHAS. H., (738)
DURAN, RUBEN, 115

EARHART, ELLEN F., (293)
EDGINGTON, L. V., (745)
EDWARDSON, JOHN R., (333)
EIDE, CARL J., (128)
ELSAID, HANY M., 852
ENGLISH, HARLEY, 688
EPPS, JAMES M., 48
EPSTEIN, A. H., 17, 216

FASSULIOTIS, GEORGE, 61
FEDER, WILLIAM A., 872
FELDMAN, A. W., 430
FELLOWS, HURLEY, 846
FENNER, LAWRENCE M., 383
FERRIS, JOHN M., 484
FERRIS, VIRGINIA R., 181
FOOTE, F. J., (267)
FORBES, IAN, Jr., (333)
FORD, RICHARD E., 655, 784
FRAZIER, N. W., 303, 547
FREDERIKSEN, R. A., 813
FREY, K. J., (88)
FRIDLUND, PAUL R., 703
FULTON, N. D., (168)
FUTRELL, MAURICE C., (835)

GASKIN, T. A., 724
GILL, TEJPAL S., (617)
GILLESPIE, WILLIAM H., (734)
GILMER, R. M., 356, 583
GINNS, JAMES H., 734
GONZALES, OSWALDO F. VARGAS, (171)
GOODNO, R. W., (404)
GORE, U. R., (438)
GORSLINE, GEORGE W., (657)
GRAHAM, J. H., (729)
GRAHAM, S. O., (722)
GRIFFIN, G. D., (17)
GROGAN, C. O., (8)
GUDAUSKAS, ROBERT T., 498

HAASIS, FRANK A., 328
HALFON-MEIRI, A., (790)
HALLIWELL, ROBERT S., 30, 220, 555
HANKS, R. W., (430)
HANNON, CHANCELLOR I., 451
HARDING, PAUL R., Jr., 100, 804
HARMON, DANIEL L., (299)
HARRISON, JAMES M., (401)
HARRISON, M. B., (631)
HARRISON, M. D., 128
HARVEY, A. E., 593
HEBERT, T. T., (438)
HEDDEN, ORVE K., (186)
HEINIS, JULIUS L., 200
HELTON, A. W., 45, 77, 325, 523, 528, (593)
HENDRIX, FLOYD F., Jr., 674
HENNEBERRY, T. J. (610)
HENSON, LAWRENCE, (372)
HEPTING, GEORGE H., 570
HEUBERGER, J. W., 159
HIKIDA, H. ROBERT, (310)
HILBORN, M. T., (80), (84)
HILDEBRAND, E. M., 796
HIMELICK, E. B., 86
HINDS, THOMAS E., 57
HINE, RICHARD B., 643
HINGORANI, M. K., (481)
HOFF, JOHN K., 24
HOFFMAN, J. R., 114
HOLMES, FRANCIS W., 715
HOLTON, C. S., 728
HOOKER, A. L., 9, 14
HOPEN, H. J., 93
HOPPE, PAUL E., 444
HORN, N. L., (185), 591, 762
HOUSTON, BYRON R., (748)
HUNTER, J. H., (894)
HUSAIN, AKHTAR, 672
HYRE, R. A., 393

INMAN, ROBERT E., 827

JACKSON, CURTIS R., 312, 888
JENKINS, ANNA E., (726)
JENKINS, SAMUEL F., Jr., 825
JENKINS, W. R., (472), (712), 858
JENSEN, HAROLD J., (699), 702
JHOOTY, J. S., 218
JOFFE, A., 203

JOHNSON, D. E., (635), 742
JOHNSON, E. M., (36), 372, 537, 539
JOHNSON, H. A., Jr., (547)
JONES, E. D., (601)
JONES, R. K., (159)
JORGENSEN, P. S., (547)

KALOOSTIAN, GEORGE H., 292
KAMALSKY, L. R., (583)
KAMP, INEZ N., (459)
KEENAN, G. I., (844)
KEIL, HARRY L., 397
KenKNIGHT, GLENN, 287
KENNEDY, B. W., 360
KILPATRICK, R. A., (346)
KIM, CHONG CHON, (435)
KING, THOMAS H., (360), 772, 777
KINMAN, M. L., (248)
KIRKPATRICK, H. C., 628, 722
KIRKPATRICK, J. D., 267
KLISIEWICZ, JOHN M., 748, 849
KLOTZ, L. J., 606
KNOX, K. A., (340)
KRAMER, C. L., 189
KRIEGER, F. C., (194)
KRULL, CHARLES F., (866)
KUHLMAN, E. GEORGE, (674)
KULKARNI, N. B., (381), (874)

LEAF, A. L., (285)
LEAR, BERT, 631, 635, (742)
LEE, KWANG YUN, 435
LEONE, IDA A., 140, (792)
LEUCK, D. B., 240
LEWIS, F. H., (163)
LINDNER, R. C., 59, (722)
LISK, D. J., (844)
LISTER, R. M., (770)
LITTON, C. C., (36)
LIVINGSTON, R. L., (831)
LOEGERING, WILLIAM Q., 299
LONG, RAY A., (692)
LOPEZ F., LUIS CESAR, 785
LORING, LINDSEY B., (702)
LUEPSCHEN, N. S., 41
LUTTRELL, E. S., 261, 661

MacLEOD, JOHN, (393)
MacSWAN, IAIN C., (30)
MAGGENTI, A. R., (303)
MAI, W. F., (24), (631)
MANKAU, R., 375
MARLATT, R. B., 428
MARTIN, ALBERT, (171)
MARTIN, W. J., 19, 21
MAY, CURTIS, (156), 384
McCAIN, ARTHUR H., 203
McCRUM, R. C., 80, 84
McCUTCHEN, B. E., 168
McDANIEL, A. T., 364
McFADDEN, LORNE A., 551
McGRATH, HILDE, 205
McKAY, HAZEL H., 26
McKEEN, W. E., (218)
McKITTRICK, R. T., (428)
McLEAN, D. M., 5, 565, 877, 882
McNICHOLAS, F., (844)
McWHORTER, FRANK P., 349
MEADER, E. M., (130)
MEREDITH, DONALD S., 305
MILBRATH, J. A., (243), (555)
MILLER, ORSON K., Jr., 576
MILLER, P. M., (258), 745
MILLER, PAUL R., 130
MILLER, P. W., (119), (220), 371
MINK, G. I., (623)
MINTEER, R. J., (375)
MINTON, NORMAN A., 199
MINZ, G., 531
MOHAMED, HOSNI A., 18, 801
MOORE, J. N., 190
MOREHART, A. L., (227)
MOREY, HOWARD R., (551)
MORGAN, G. T., 800
MORRIS, C. L., 340
MORTENSEN, J. A., (446)
MURRAY, B. R., (261)

NEELY, DAN, (86)
NEFF, T., (269)
NELSON, MERRITT R., 667
NEWHOOK, F. J., (570)
NICHOLS, LESTER P., 172
NICOLSON, T. H., (616)
NILSSON, GOSTA I., 152

O'BANNON, JOHN H., 558
OGAWA, JOSEPH M., 614
OLD, K. M., 616
OLSON, E. O., (882)
ORCHARD, W. R., (92)
ORELLANA, R. G., (248)
ORJUELA, JUAN, 866
ORTON, E. R., (320)
OSBORNE, W. W., 712
OTEIFA, BAKIR A., 572
OWEN, J. H., (831)

PALTI, J., (145), (418)
PAPAVIZAS, G. C., 646
PARKER, K. G., (404), 844
PARMETER, J. R., 269
PARRIS, G. K., 677, 682
PARSONS, J. L., (597)
PATEL, G. J., 173
PEABODY, DWIGHT V., Jr., (256)
PETERSON, J. L., 277, 290
PFAELTZER, HILLEGONDA J., 338

PHILLIPS, DOUGLAS J., 506
PINE, T. S., 495
PLAKIDAS, A. G., 150
POINTER, GERALD R., (883)
POLLARD, H. N., (292)
PROTACIO, D. B., 686
PURDY, LAURENCE H., (728)

RANNEY, C. D., 122
RAO, VASANT GURUNATH, 381, 874
RASKI, D. J., 516
RAU, G. J., (441)
RAWITZ, E., (145)
RAWLINS, T. E., 170
REDIT, W. H., (221)
REYES, LUCAS, 835
REYNOLDS, HAROLD W., (558)
REYNOLDS, J. E., 243
RHOADES, H. L., 424
RICH, AVERY E., 130, (346), (839)
RICHARDS, H. RANDOLPH, 818
RICHARDS, M. C., (130)
RISLEY, J. R., (285)
RIVERA, CARMEN M., (758)
RIVERS, G. W., (248)
ROBERT, ALICE L., 321
ROBERTSON, R. S., Jr., 71
ROSINSKI, MARTIN, (265)
ROSS, J. P., 766
ROTEM, J., 145
RUEHLE, JOHN L., 710

SABET, K. A., (481)
SAHO, HARUYOSHI, 34
SAMRA, A. S., 481
SANDENO, JAMES L., 699
SANTIAGO, ALMA, (758)
SASSER, J. N., 171, (328), (640)
SAVAGE, DONALD C., (804)
SCHENCK, N. C., 446
SCHIEBER, EUGENIO, 202, (857)
SCHINDLER, A. F., 610
SCHMIDT, R. A., (194)
SCHMITTHENNER, A. F., 597
SCHOEN, JAMES F., 540
SCHREIBER, LAWRENCE R., 401
SHAFIEE, M. FAROUK, 472
SHAH, H. M., (173)
SHATLA, M. N., (744)
SHIGO, ALEX L., 379
SILBER, GUSTAVE, 137
SILBERNAGEL, M. J., (784)
SILVERBORG, S. B., 285
SILL, W. H., Jr., 780
SIMONS, M. D., 88
SINCLAIR, J. B., 185, 744, (852)
SITTERLY, W. R., 492
SLEDGE, E. B., 52, 735
SMALLEY, E. B., (216)
SMART, GROVER C., Jr., 65
SMITH, A. L., (462), (609)
SMITH, FLOYD F., (335)
SMITH, HARLAN E., 462, 609
SMITH, M. A., (41)
SMITH, ROY J., 487
SMITH, W. L., Jr., 221, 861
SMITHSON, HARRIET R., (702)
SOWELL, GROVER, Jr., 883
STALL, R. E., 389
STAMBAUGH, W. J., 194
STEELE, ARNOLD E., 43, 417
STEVENSON, JOHN A., 131, 204, 230
STEWART, LINNEA, 469
STODDARD, E. M., 258
STOKER, GOLDEN L., 176
STOLZY, L. H., (606)
STONE, W. J., 623
STONER, WARREN N., 38
STOVER, L. H., (446)
STRETCH, A. W., (31)

TABOADA, O., (114)
TALENS, LUCIANO T., (780)
TAMBURO, S. E., 281
THAKUR, RAM NATH, (672)
THAYER, P. L., (389)
THEIS, T., (105), (758)
THOMAS, C. A., 248
THOMAS, H. E., (547)
THOMAS, JOHN E., 463
THOMAS, WALTER I., (657)
THOMPSON, HUGH E., (189)
THORNBERRY, H. H., 456, 459
THORNE, GERALD, 857
THURSTON, H. DAVID, (866)
TOBA, H. HAROLD, 409
TODD, EDWIN H., 486
TOOLE, E. RICHARD, 732
TRUJILLO, E. E., 819
TSAO, PETER H., 357
TULLIS, EDGAR C., 64
TURNER, W. F., (292)
TUTTLE, DONALD M., (667)

VALLEAU, W. D., 36, (372), 461
VAN ARSDEL, E. P., 306
VAN PELT, HOWARD, (348)
VARNEY, E. H., (190)
VAUGHAN, EDWARD K., (310), (342)
VLAMIS, J., 886
VOLCANI, ZAFRIRA, 175, 893
von RUDEN, DALE A., (772)

WALKER, JERRY T., 293
WALLACE, JAMES M., 211, (414)
WASEEM, MUHAMMED, 586
WATSON, ALICE J., 726
WEBB, R. E., 260

WEHLBURG, C., 98
WELCH, J. E., (719)
WELLS, HOMER D., (240), 333
WELLS, J. C., 65
WERNHAM, C. C., 657
WESTER, R. E., 617
WHITE, L. V., (501)
WILBUR, W. D., (750)
WILSON, CHARLES L., (364), 448
WILSON, J. D., 124, 186
WILSON, ROY A., (397)
WINTER, H. F., 560
WITCHER, WESLEY, 64
WOOD, G. W., (800)
WYSONG, DAVID S., 274

YARWOOD, C. E., 317, 755, (886)
YATES, WESLEY E., (614)
YOUNG, P. A., 151
YOUNG, WENDELL, (750)
YOUNG, WILLIAM J., 750
YOUTSEY, CHARLES O., (279)

ZEIDERS, K. E., 729
ZIMMER, D. E., 665
ZINK, F. W., 719

SUBJECT INDEX

Abies sp.: Indian paint fungus, study of the germination of the basidiospores, their viability and early growth of their germ tubes, 576
Achras sapota: Phytophthora palmivora, 1st rept. on this host (India), 381
African violet: bacterial leaf blight, new disease of, 30
Agrobacterium tumefaciens, control with soil fumigants, 73
Agrostis tenuis: bentgrass nematode, effect of herbicides on development of, 256
Air pollutants (see also Ozone), phytotoxic, correlation with meteorological phenomena, in New Jersey, 140
Air pollution, possible cause of chlorotic decline of ponderosa pine in southern California, 269
Alabama, 232, 498, 710
Alfalfa: Ditylenchus dipsaci, 1st rept. from Peru, 171; Meloidogyne spp., resistance of 13 breeding lines of African and one Sirsa selection, 558; seedling losses, relationship of preemergence and postemergence, 597
Allium canadense: Pratylenchus penetrans, source of resistance, 535
Almond, see Prunus
Alternaria blight, of rape and mustard in India, control with griseofulvin, 672
--- brassicae, 672
--- citri, 200
--- dianthi, 152
--- dianthicola, 152
Amaranthus palmeri: tobacco ringspot virus, 1st rept. on this host (Texas), 5
--- retroflexus: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Amaryllis sp.: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
Ammonia injury, to apples and peaches in storage, 792
Ammonium bifluoride, stump protectant against Fomes annosus, 340
Anguina agrostis, in bentgrass, effect of herbicides on development of, 256
Angular leaf spot, of tobacco, 36
Annona squamosa: Phytophthora parasitica var. macrospora, 1st rept. from India, 874
Announcement: request for specimens of Fusarium tuber rot of potato, 205
Anthracnose, on strawberry in Louisiana, 591; of tomato, control, 227; of watermelon, 41
Antibiotics (see also Fungicides, etc.): for control of blister rust on white pine, 306
---: cycloheximide, for control of hawthorn leaf blight, 827
---: griseofulvin, for control of Alternaria blight of rape and mustard, in India, 672
---: streptomycin, resistance and susceptibility of tomato bacterial spot pathogen, 389
---: streptomycin-oxytetracycline, for control of fire blight on Bartlett pear, 397
Aphanomyces euteiches, 646, 777
--- root rot, of peas, control, 646
Aphelenchoides besseyi, 293
--- fragariae, in western swordfern, 699
Aphis medicaginis, 240
Apparatus, see Techniques and apparatus
Apple: ammonia injury in storage, 792
---: black pox, 1st rept. in Oregon, 30
---: blotch, control with fungicides in South Korea, 435
---: dapple apple (virus), 80
---: dinitro-ortho-cresol, surface and internal residue analysis of fruit, 844

---: effect of fungicides on fruit numbers and yields, 560
---: Elsinoë piri, 1st rept. in Germany (?), Poland and USSR, 726
---: flat limb (virus), 80
---: latent viruses, rapid detection on Hopa crab, 243
---: mosaic (virus), 80
---: nematode populations, associated with poor terminal growth in West Virginia, 281
---: scab, control studies in Delaware, 159, in Pennsylvania, 163
---: scald and Stayman spot, chemical dips for control, 808
---: sooty blotch in New Jersey, control, 513
---: stem pit (virus), 80
---: virus diseases (dapple apple, flat limb, mosaic, and stem pit), comparative symptoms, 80
Arizona, 156, 428, 558, 667
Arkansas, 168, 364, 448
Ascochyta leaf spot, on winter oats in Kentucky, 818
Aspergillus crown rot, of peanut in Georgia, 888
--- niger, 888
--- spp., in stored bean seed, 785
Azalea: stem canker, 1st rept. in Oregon, 30

Bacillus polymyxa, in Israel, 893
--- subtilis, effect of plant growth regulators, 469
Bacteria, effect of plant growth regulators, 469
Bacterial angular leafspot, of strawberry, epidemiology studies, 360
--- blight, of cotton in Arkansas, 168
--- leaf blight, of inbred corn lines, comparative resistance, 277
--- leaf spot, of pepper in Florida, 652, of poinsettia, 1st rept. in U.S. (Florida), 551
--- leaf stripe, of corn in Florida, 98
--- necrosis, of saguaro in Arizona, 156
--- spot, of peach, control, 251; of tomato and sweet pepper, in Israel, 175, streptomycin-resistant and -susceptible isolates, 389
--- wilt, of inbred corn lines, comparative resistance, 277
Bacteriocide (see also Fungicides, etc.): dodine-sulfur combination for control of bacterial spot of peach, 251
Banana: eyespot, new foliar disease, in Jamaica, 305
--- mosaic outbreak in Honduras, means of control, 366
---: pitting disease, on Chicago and Milwaukee markets, 1st rept. on Central American bananas, 354
---: Radopholus similis, 1st rept. from Peru, 171
---: Rotylenchulus reniformis, 1st rept. from South America (Peru), 171
---: Sigatoka leaf-spot disease in Puerto Rico, effect of climatic factors, 758
Barley: Glomerella cingulata, seed borne, 1st rept., 111; Myrothecium roridum, Myrothecium verrucaria, seed borne, 1st rept., 111; powdery mildew, effect of liming of soil on, 886
Basal canker disease, of eastern white pine in New York, 285
Bean: Fusarium solani f. phaseoli, 1st rept. in Hawaii, 819
---: mosaic diseases, epidemiology studies, role of seed transmission, 71
---: Rhizoctonia solani in Israel, value of commercial seed grading, 790
---: storage fungi, invasion of and damage to seed, 785
---: tobacco mosaic virus, reaction to tomato and tobacco strains of, 537
---: Uromyces phaseoli typica, 15 new races in Mexico, 411
---, lima: downy mildew in New Jersey, forecasting methods, 393, strain "B", spread in New Jersey, 617
---, snap: Rhizoctonia solani in Florida, chemical control, 651; Sclerotium rolfsii in Florida, chemical control, 651
Belonolaimus longicaudatus, effect on St. Augustine grass, 424; on grape, 1st rept. on this host (Florida), 446; on peanuts, control with Cynem, 640
Birdsfoot trefoil see Lotus corniculatus
Black root rot complex, of strawberry, effect of Pratylenchus penetrans, 839
Black rot, of sweetpotato, control, 253, of watermelon, 41
Black shank, of tobacco, lab method for estimating disease potential index in infested soil, 825
Black spot, of citrus in South Africa, temperature as a control factor, 487
Blister rust, on white pine, control, 306
Blossom-end rot, of watermelon, 41
Blotch, of apple in South Korea, control, 435
Blueberry (low-bush): Pratylenchus penetrans, nonpathogenic to, 84
Boerhaavia coccinea: tobacco ringspot virus, 1st rept. on this host (Texas), 5
--- erecta: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Book reviews: "A Monograph of the World

(Book reviews) Species of Hypoxylon" by Julian H. Miller, 131; "Color Handbook of Citrus Diseases" by Leo J. Klotz, 130; "Fungicides in Agriculture and Horticulture", 205; "How to Control Plant Diseases in Home and Garden" by Malcolm C. Shurtleff, 294; "The Genus Tilletia" by Ruben Duran and George W. Fischer, 204
Botryosphaeria sp., undescribed, causing twig blight of Arizona cypress in Georgia, 261
Botrytis cinerea, 260, 342
--- inflorescence blight (undescribed disease), of holly in New Jersey, 320
Brachiaria mutica: chlorosis due to iron deficiency in Nigeria, 38
Brassica campestris: Alternaria blight, control with griseofulvin, in India, 672
Bremia lactucae, 719
British Columbia, 92
Broccoli: downy mildew, in Pacific Northwest, control tests with negative results, 310
Bromus inermis: ergot in Michigan, 838; Stagonospora bromi in Pennsylvania, 729
Brown rot, of peach, control, 31, 221, 476, 861
Bud-perpetuated conditions in prune trees, relationships and variations, 523
Bud-union disorder, of Eureka lemon on No. 1452 citrumelo rootstock, 267
Buxus sempervirens var. arborescens: Pratylenchus vulnus, pathogenicity studies, 712

Cabbage, fodder: bacterial soft rot of collar and stem, in Israel, 893
California, 107, 115, 203, 211, 269, 357, 516, 547, 614, 688, 719, 748, 750, 849
Calla sp.: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
Camellia: flower blight, in Mississippi, 617
Canada, 218
Cantaloup: crown blight complex, role of mosaic diseases in Arizona, 667
Carica papaya: tobacco ringspot virus, possible cause of papaya decline in Texas, 882
Carnation: wilt diseases, in Sweden, 152
Carnegiea gigantea: bacterial necrosis, in Arizona, 156
Carrot: soil treatments, effect on yields and flavor, 631
Carthamus sp.: powdery mildew in California greenhouse, 203; Verticillium wilt, potential problem in U. S., 665
--- tinctorius: cucumber mosaic virus, in California, 849; Fusarium wilt, 1st rept. on this host (Calif.), 748
Carya ovata: witches' brooms and galling, in Arkansas, 448
Caryopteris: root knot, relationship to winter survival, in Ohio, 186
Celery: soil-borne pests in seedbeds, control studies, 441
Cenangium singulare, causal agent of sooty-bark canker of aspen, 57
Cephalosporium sp., 379
Cephalosporium sp. (?), possible cause of new wilt disease of corn in Egypt, 481
Cerastium tomentosum: Rhizoctonia sp., 1st rept. on this host, (Mass.), 172
Ceratocystis coerulescens, infects and invades freshly cut hardwood logs, 379
--- fimbriata, 253
--- ulmi, 86, 189, 216, 401, 463
Cercospora apii, 461
--- capsici, 652
--- leaf spot, of pepper in Florida, 652
--- spp., oil sprays for control, 105
Chemical agents, in vitro effects on Cytospora cincta, 593
--- dips, for control of scald and Stayman spot of apples, 808
Chemicals: amino acids, for control of Aphanomyces root rot of peas, effectiveness increased by combination with fertilizer, 646
---: calcium nitrate, for control of tomato southern blight, 492
---: evaluated for systemic therapeutic action against Radopholus similis in citrus seedlings, 430
---: EF 1261-960, for control of soil-borne and foliage diseases of pepper in Florida, used as soil treatments and foliar sprays, 652
---: plant growth retardants and germicides, effect on bacteria, 469
Chenopodium amaranticolor: tobacco ringspot virus, 1st rept. on this host (Texas), 5
--- murale: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Cherry: green ring mottle virus, not seed-transmitted to mazzard seedlings, 356; necrotic ring spot and sour cherry yellows, in commercial seedling root-stocks in N. Y., 583; sour cherry yellows virus, counteracting influence with gibberellin sprays, 404

---, sweet: crinkle leaf and deep-suture symptoms, comparative studies, 325
Chloropicrin injury, to sweetpotatoes, 246
Chlorotic decline, of ponderosa pine in southern California, drought or air pollution possible causes, 269
Citrus aurantifolia: "tatter leaf", undescribed virus, in California, 211
--- excelsa: "tatter leaf", undescribed virus, in California, 211
--- limon: bud-union disorder, incompatibility with No. 1452 citrumelo rootstock, 267
--- paradisi: Radopholus similis, movement from infected to non-infected potted seedlings, 735
--- sp(p.): black spot in South Africa, low temperature as a control factor, 487; Geotrichum candidum and Trichoderma viride, biphenyl treatment in relation to nonvented and vented cartons, 804; green mold strains, differential sensitivity to sodium orthophenylphenate, 100; leaf spot on Rangpur lime and related species in Texas, control, 200; Penicillium digitatum, differential reaction of isolates to biphenyl vapor and effect on green mold control, 115; Radopholus similis infected seedlings, host-pathogen response to chemicals, 430; sour rot, in Louisiana, 185; Thielaviopsis basicola, prevalent in California orchards, 357; Tylenchulus semipenetrans, occurrence and distribution in Florida, 451
Cladosporium carpophilum, 476
--- cucumerinum, 93
--- herbarum, inhibits gray mold rot of strawberries, 342
--- sp(p.), 614; associated with needle cast of larch and spruce, 1st rept. in N. A. (Maine), 265; of tomatoes, in Oregon, 30
Claviceps purpurea, 838
Clerodendrum thomsoniae: zonate ringspot, new virus disease, in Florida, 279
Cocoa: Rotylenchulus reniformis, 1st rept. from South America (Peru), 171
Coconut: cadang-cadang, intracellular inclusion bodies observed in foliage epidermal strips, 686
Coffee: Xiphinema americanum in Guatemala, means of control, 857
Colorado, 57, 506
Colletotrichum fragariae, 591
--- lagenarium, 41
--- phomoides, 227
Colombia, 866
Control, biological, of gray mold rot of strawberries, 342
Control program, of banana mosaic in Honduras, 366
Corn: bacterial wilt and leaf blight, comparative resistance of inbred lines, 277
---: crazy top (Sclerospora), 1st rept. in Mississippi, 8;
"giantism" a new symptom, 8
---: Fusarium root rot, in Israel, 203
---: Helminthosporium spp., in relation to yield reduction and stalk rot severity in hybrids, in Pennsylvania, 657
---: northern leaf blight, winter survival studies in Wisconsin, 444
---: Puccinia sorghi, 26 additional sources of resistance, 14
---: stalk rot, in Egypt, 18; relation of Helminthosporium leaf blights to severity of, 657; yield reduction highly significant in Illinois, 9
---: wilt disease, new, in Egypt, 481
---, sweet: bacterial leaf stripe in Florida, 98
Correction, 384, 750, 819
Coryneum beijerinckii, 614, 688
--- blight, of almond, control, 614; of peach in California, control, 688
Cotton: bacterial blight, distribution of races in Arkansas, 168
---: diseases, reduction in yield caused by, 1957-1961, 609, in 1961, 462
---: fungi and other microorganisms isolated from seedlings, effect of planting date in Egypt, 801
---: Fusarium wilt in alkaline soil in Mexico, 396
---: Meloidogyne incognita-seedling disease complex, interactions, 501
---: Rotylenchulus reniformis, 1st rept. from South America (Peru), 171
---: seed delinting, Taylor's pioneer role, 230
---: seedling disease complex, fungi involved in the Yazoo-Mississippi Delta, 122
---: sore-shin, greenhouse technique for evaluating fungicides for control, 852
---: Stemphylium leaf spot, 1st rept. in Louisiana, 744
Covered smut, of crabgrass, 64
Crataegus oxyacantha vars.: leaf blight, control under nursery conditions, 827
Crazy top, of corn in Mississippi, 8

Criconemoides xenoplax, in soil around canker-diseased spruce in Wisconsin, 17
Crinkle leaf, of sweet cherry, 325
Cronartium fusiforme, 64
--- ribicola, 306
Cropping sequences, increased nodulation of soybeans in soybean-cyst-infested soil, 48
Crown blight complex, of cantaloup in Arizona, 667
--- rust, of oats in texas, new races, 835
--- ---, race 264, attempt to induce resistance in oats by irradiated seed, 88
Cucurbits: Bacillus polymyxa on Honey Dew melons, in Israel, 893
---: citrus psorosis virus (infectious variegation strain) transmitted mechanically to cucumber, 414
---: cucumber scab, inhibited by mosaic, 93
---: diseases of watermelon on Chicago market, 41
---: gummy stem blight, resistance among introductions, 883
---: mosaic virus of cucumber, effect on development of scab, 93
---: watermelon mosaic virus, host range studies, transferred to wild and cultivated Cucurbitaceae only, in Hawaii, 409
Cuerna costalis, 292
Cunninghamia: necrotic leaf spot, 1st rept. on this host (Oregon), 30
Cupressus arizonica: Botryosphaeria (undescribed species) twig blight in Georgia, 261; Phomopsis juniperovora, in Arkansas, symptoms and control studies, 364
Curvularia trifolii, 312
Cydonia vulgaris: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
Cynodon dactylon: Helminthosporium spiciferum, 1st rept. of pathogenicity on this host (Ala.), 498; Sclerotinia blight, in Mississippi, 55
--- transvaalensis: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
Cytospora canker, of prune trees, 45; on spruce in Wisconsin, 17
Cytospora cincta, in vitro effects of chemical agents, 593
--- sp(p.), on Pieris japonica (new host), 30; on prune, factors affecting invasion, 77

Damping off, in tobacco plant beds in Kentucky, 539
Decline, of pear in Greece, similar to American pear decline and Italian moria, 150
Decline, sudden, of peach trees in Georgia, 831
Deep suture, of sweet cherry, 325
Deficiency diseases: iron deficiency of grasses in Nigeria, 38
Delaware, 159
Digitaria ischaemum: covered smut, in Maryland, 64
Dioscorea sp.: nematodes associated with, in Guatemala, 858
Diplodia natalensis, 41
--- zeae, 9
Diseases, losses and crop value correlation, 755; reduction in yield of cotton caused by, 1957-1961, 609, in 1961, 462
Distribution, of the citrus-root nematode in Florida, 451; of recorded Dutch elm disease in North America as of 1961, 715
Ditylenchus dipsaci, in alfalfa, 1st rept. in Peru, 171; in garlic cloves, treatments for eradication, 635
Dolichodorus (undescribed species), in soil from around a mahogany tree, 1st rept. from South America (Peru), 171
Downy mildew, of broccoli in Pacific Northwest, 310; of lima bean in New Jersey, forecasting incidence of, 393, strain "B", spread in New Jersey, 617; of peas, prevalent in Pacific Northwest in 1962, 784; of susceptible lettuce, factor in post-harvest deterioration, 719
Drechslera gigantea, on banana, in Jamaica, 305
Drought injury, possible cause of chlorotic decline of ponderosa pine in southern California, 269
Dutch elm disease, 216; control studies, negative results, 401; 1st rept. in Oklahoma, 463; recorded distribution in North America, 715; root grafting of city-planted elms, 86; spread in Kansas, 189

Echinodontium tinctorium, study of the germination of basidiospores, their viability and early growth of the germ tubes, 576
Economic crops: Pratylenchus spp., occurrence in Nile Delta Valley, 572
Egypt, 18, 481, 801
El Salvador, 293
Elsinoë lepagei, on Pouteria campechiana, 1st rept. in Mexico, 726
--- piri, on Malus sylvestris, 1st rept. in Germany (?), Poland and USSR, 726

Entomosporium sp., on Raphiolepis indica (new host), 30
Epidemic, of tomato ghost spot in Maryland, 260
Epidemiology, of bacterial angular leafspot on strawberry in Minnesota, 360; of bean mosaic diseases, 71
Epiphytotic, of sour rot of citrus in Louisiana, 185
Ergot, on smooth bromegrass in Michigan, 838
Erwinia amylovora, 397, effect of plant growth regulators, 469
--- carnegieana, 156
--- carotovora var. aroideae, on cruciferous hosts in Israel, 893
Erysiphe cichoracearum, 203
--- graminis, 724, 886
Escherichia coli, effect of plant growth regulators, 469
Ethylene oxide, for sterilization of wheat seed, 540
Euphorbia cyparissias: Rhizoctonia sp., 1st rept. on this host (Mass.), 172
--- pulcherrima: bacterial leaf spot, 1st rept. in U. S. (Florida), 551; Sphaceloma poinsettiae, 1st rept. in Arizona and Texas, 726
Eyespot, of banana, new foliar disease, in Jamaica, 305

Fabraea maculata, 827
Fertilizer, in combination with amino acids, effectiveness for control of Aphanomyces root rot of peas, 646
Festuca elatior var. arundinacea: Rhizoctonia solani, perfect stage of, in Georgia, 661, isolates, pathogenicity and resistance studies on, in Georgia, 661
Ficus spp.: fig mosaic virus, additional hosts, 693
Filbert: kernel-spot, 1st rept. in U. S. (Oregon), 220
Fire blight, of Bartlett pear, control, 397
Flax: aster yellows, effect of systemic insecticides, in Minnesota, 813
Florida, 52, 98, 279, 348, 424, 430, 441, 446, 451, 486, 551, 651, 692
Flower blight, of camellia, in Mississippi, 617
Fomes annosus, 734; causing death of pines in California, 107; conks on slash pine in South Carolina, 64; lab method of obtaining sporophores 63; on Pinus echinata, methods of isolation, 674; on shortleaf pine in Missouri, 521; on slash pine stumps in south Georgia, 738; on white pine in Pennsylvania, 194; role of conidia in establishing infection, 340
Forecasting, downy mildew of lima bean in New Jersey, 393
Forecasting system, for phytotoxic air pollutants, basis for control measures, 140
Fragaria (see also strawberry)
--- chiloensis: powdery mildew, 1st rept. from Florida, 348
--- vesca: latent A virus, excluded by apical meristem cultures and heat treatment of parent plants, 119
--- virginiana: latent A virus, effective indicator host for, 772
Frogeye, of tobacco in Venezuela, 461
Fruit rot, of tomato, control, 227
Fuchsia: root and crown rot, new disease of, 30
Fungi, associated with basal canker disease of eastern white pine, 285, with gladiolus roots, 312, with wilt diseases of carnations, in Sweden, 152
---, infecting hardwood bolts, 379
---, involved in the seedling disease complex of cotton, in the Yazoo-Mississippi Delta, 122
---, isolated from cotton seedlings in Egypt, 801
---, method for determining the oxygen requirement in liquid media, 606
---, new survival records of several fungi preserved on agar under mineral oil seal, 192
---, pathogenic to forest trees introduced in Japan, check list, 34
---, imperfect, in slash pine stumps in south Georgia, 738
---, nematode-capturing, collected in North Carolina, 872
---, secondary (see also secondary organisms), associated with pitting disease of banana, 354
---, soil, use of nylon mesh in studies of, 616
---, storage, invasion of and damage to bean seed, 785
Fungicide spray programs: effect on fruit numbers and yields of apple trees, 560
Fungicide sprays, for control of almond diseases, effect of operating techniques and application equipment, 614
Fungicides (see also antibiotics, herbicides, insecticides, nematocides, etc.)
---: Dexon, effective in controlling Phytophthora crown rot of petunia, 506
---: 2, 6-dichloro-4-nitroaniline, effec-

(Fungicides) tive for control of postharvest peach decays, 31
---: dinitro-ortho-cresol, residue analysis in apple, 844
---: Dyrene, for control of Stemphylium leaf spot of birdsfoot trefoil, 509
---: effect on cotton seedling disease, root knot, and plant growth, 501
---: ferbam and ziram, most effective in comparison tests for control of Coryneum blight of peach in California, 688
---: for control of apple blotch, 435; apple scab, 159, 163; black rot of sweetpotato, effect on total yields, 253; cotton sore-shin, greenhouse technique for screening, 852; downy mildew of broccoli, negative results, 310; leaf spot on Rangpur lime, 200; needle blight on Arizona cypress, 364; pecan scab in Ala., increased returns in quality and production, 232; pecan scab in Oklahoma, 236
---: sodium orthophenylphenate, differential sensitivity of biphenyl-resistant and -sensitive strains of Penicillium digitatum to, 100
Fungicides-additives: for control of brown rot and scab of peach, 476
Fungicides-insecticides: for control of sooty blotch of apple, 513
Fungicides, new soil: CP-30249 and DAC-649, for control of scab and Rhizoctonia, 601
Fungicides, postharvest: for control of peach decays, 221
Fungicides, soil drenches: effect on Rhizoctonia root disease and two-spotted spider mite on strawberry, 258
Fusarium moniliforme, 203
--- oxysporum, 748
--- --- f. apii, of parsley, in Oregon, 30
--- --- f. conglutinans, 124
--- --- f. dianthi, 152
--- --- f. lycopersici, 151
--- --- f. vasinfectum, 396
--- root rot, of corn in Israel, 203
--- roseum, 152
--- solani, on swamp tupelo in Louisiana, 732
--- --- f. phaseoli, on bean, 1st rept. in Hawaii, 819
--- spp., 122, 312
--- wilt, of safflower, 1st rept. (Calif.), 748; of tomato in Texas, control, 151; of Upland cotton in alkaline soil in Mexico, 396
Fusicladium effusum, 232, 236; 1st rept. of survival on agar under oil, 192

Garlic: black scurf, 1st rept. in Oregon, 30; Ditylenchus dipsaci, treatments for eradication, 635
Georgia, 261, 292, 438, 625, 661, 710, 738, 831, 888, 894
Geotrichum candidum, 185; of lemon, spread by contact infection in nonvented versus vented cartons, 804
Ghost spot, of tomato in Maryland, 260
Gibberella zeae, 9
Gladiolus: cowpea mosaic viruses, isolated from, 335; root-infecting fungi, 312; viruses, transmitted on tools in harvesting flowers and corms, 505
Gliocladium roseum, 379
Gloeodes pomigena, 513
Gloeosporium quercinum, tentatively identified, on oaks in Mississippi, 677
--- sp., of azalea, in Oregon, 30
Glomerella cingulata, seed borne in small grains, 1st rept., 111
--- gossypii, 122
Gloxinia: bacterial leaf blight, new disease of, 30
Gomphrena globosa: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Gramineae: new hosts for Helminthosporium spp. from corn, 321
Grape: Belonolaimus longicaudatus, 1st rept. on this host (Florida), 446; nematode control, DBCP experiments in established vineyards, 516; Pierce's disease virus, transmitted by leafhopper vectors, in Georgia, 292
Gray mold rot, of strawberries, biological control studies, 342
Greece, 150
Green mold, of citrus, 100, 115
Growth-regulating substances: effect on bacteria, 469; CCC, effect on tobacco mosaic virus, 170; gibberellin, effect on bud differentiation of sour cherry and on damage by sour cherry yellows virus, 404
Guatemala, 202, 857, 858
Gummosis, of peach in Colorado, 274
Gummy stem blight, of watermelon, 883
Gymnosporangium clavipes, on medlar, 1st rept. in U.S. (Ind.), 293

Hardwood bolts, succession of fungi attacking, 379
Hawaii, 409, 643, 819
Hawthorn: see Crataegus
Helicotylenchus dihystera, 710
--- erythrinae, 710
--- sp., on lowbush blueberry in Mari-

(Helicotylenchus) time Provinces, 800
Helminthosporium allii, of garlic, in Oregon, 30
--- carbonum, races 1 and 2, new Gramineae hosts for, 321
--- maydis, new Gramineae hosts for, 321
--- papulosum, of apple, in Oregon, 30
--- spiciferum, on Coastal Bermuda grass, 1st rept. of pathogenicity on this host (Ala.), 498
--- sp(p.), cause of target blotch on sugarcane, in Florida, 486; of corn hybrids in Pennsylvania, 657; viability and cultural changes after storage under oil, 290
--- turcicum, 444; new Gramineae hosts for, 321
Hemicycliophora vidua, 710
Hendersonia rubi, 614
Herbaceous plants, indicator hosts for virus diseases of pears, 338
Herbicides, applied in a nematode-infested field of bentgrass, effects on head density and nematode development, 256; lack of influence on effect of fungicidal soil treatment for tomato fruit disease control, 227
Heterodera glycines, 48; on soybeans, physiological strains, 766
--- humuli, on hops, 1st rept. in Oregon, 702, 1st rept. in U.S. (Washington), 541
--- schachtii, emergence of larvae, effect of pretreatment of cysts with sucrose, 43, effect of removing eggs from cysts, 417
Heterosporium echinulatum, 152
--- sp., on Thuja (new host), 30
Hibiscus cannabinus: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Homalodisca coagulata, 292
Honduras, 366
Hoplolaimus galeatus, 710
Host range studies, with Rotylenchulus reniformis in Louisiana, 683, tobacco and tomato ringspot viruses, 877, watermelon mosaic virus in Hawaii, 409
Hot-water treatments, of peaches to reduce postharvest decays, 861
Humulus lupulus: Heterodera humuli, 1st rept. in Oregon, 702, 1st rept. in U.S. (Washington), 541

Idaho, 45, 325, 523, 528
Ilex aquifolium: root-lesion nematodes and poorly-drained planting sites affecting decline of, in British Columbia, 92
--- crenata: nematode and weed control with soil fumigants, economic gain in North Carolina, 328
--- opaca: Botrytis inflorescence blight (undescribed disease), in New Jersey, 320
Illinois, 9, 86, 181, 724
Incompatibility, of Eureka lemon with citrumelo rootstock, 267
India, 173, 381, 672, 874
Indiana, 293, 484, 535, 623
Indicator hosts, flowering crab vars. for fast detection of a latent virus of apple, 243; Fragaria virginiana for strawberry latent A virus, 772; herbaceous plants for virus diseases of pears, 338
Insecticides, systemic: for indirect control of bean yellow mosaic virus of yellow lupine, 240; to reduce aster yellows virus in flax, 813
Intracellular inclusion bodies, found in cadang-cadang infected foliage epidermal strips of coconut, 686
Irradiation, of oat seed not effective in inducing resistance to crown rust, 88
Irrigation methods, effect on development of Phytophthora blight on potatoes, in Israel, 145
Iris: leaf streak and stunt (virus), 1st rept. in Oregon, 30
Iron deficiency, of grasses in Nigeria, 38
Israel, 145, 175, 203, 418, 531, 790, 893

Jamaica, 305
Japan, 34

Kalanchoë flammea: mosaic, new virus disease, in Florida, 692
Kansas, 189
Kentucky, 36, 372, 539, 818
Kernel-spot, of filberts, 1st rept. in U.S. (Oregon), 220
Korea, 435

Larix decidua: Cladosporium spp., associated with needle cast, 1st rept. in N.A. (Maine), 265
Late blight, of tomato, new resistant variety, 130
Leaf blight, of almond, control, 614; hawthorn, control, 827
Leaf blister, of oaks in Mississippi, 682
Leaf scald, of rice, 1st rept. in El Salvador, 293, 1st rept. in Guatemala, 202
Leaf spot, on Rangpur lime in Texas, 200
Lettuce: big vein (virus), effects on furrow-irrigated crop, 428; downy mildew and secondary organisms, association with postharvest deterioration, 719

Light, effect on Septoria infection of wheat leaves, 846
Lilium longiflorum: cucumber mosaic virus, eliminated by scale propagation, 627; ringspot virus, in plants from Georgia, 625
Lima bean: see Bean, lima
Lime, effect on barley powdery mildew, 886
Liquid nitrogen, Puccinia graminis tritici, effect of thawing temperature on urediospores frozen in, 299
Littleleaf, of shortleaf pine, nematodes associated with, 710
Littleleaf-like disease, of shortleaf pine in New Zealand, 570
Longevity records, of fungi preserved under oil, 192, 290
Losses: from corn stalk rot in Illinois, 9; cotton diseases 1957-1961, 609, in 1961, 462; in relation to crop value, 755
Lotus corniculatus: Ramularia schulzeri, 1st rept. on this host and in New York, 655
Lotus corniculatus: Stemphylium leaf spot in Virginia, control with Dyrene, 509
Louisiana, 185, 591, 683, 732, 744, 762
Lupinus angustifolius: Stemphylium loti, highly pathogenic on breeding lines, 333
Lupinus luteus: bean yellow mosaic virus, indirect control with systemic insecticides, 240

Macrosiphum rosae, 1st rept. in Louisiana, 762
Macrosteles fascifrons, 813
Mahonia aquifolium: twig and stem blight, 1st rept. on this host (Oregon), 30
Maine, 265
Malus sylvestris: see Apple
Malvastrum coromandelianum: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Margaranthus solanaceus: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Maritime Provinces, 800
Market disease(s), of banana, 354; watermelon, 41
Marssonina mali, 435
Maryland, 64, 260, 397, 796
Massachusetts, 172
Meloidogyne hapla, on Caryopteris in Ohio, 186
--- incognita, 501
--- --- acrita, in sweetpotato, control studies, 21; resistance of alfalfa breeding lines, 558
--- --- incognita, on Dioscorea sp. in Guatemala, pathogenicity studies, 858
--- javanica, resistance of alfalfa breeding lines, 558; susceptibility of peach rootstocks, 531
--- sp., (grass root-knot nematode) host studies, 52
Mentha spicata: new disease, caused by tobacco ringspot virus, 623
Mespilus germanica: Gymnosporangium clavipes, 1st rept. on this host in U.S. (Ind.), 293
Meteorological phenomena, phytotoxic air pollutants in relation to, 140
Method (see also Technique): accurate means of testing virulence of Valsa leucostoma, 274; assay of nematocides, 742; determination of nematode mortality, 383; elimination of cucumber mosaic virus in Easter lilies by scale propagation, 627; evaluation of resistance or susceptibility of Pisum sativum varieties to Aphanomyces euteiches, 777; exclusion of latent A virus of Fragaria vesca by apical meristem culture, 119; forecasting downy mildew of lima bean in New Jersey, 393; isolation of Fomes annosus, 674; laboratory estimation of disease potential index of black-shank infested soil, 825; obtaining sporophores of Fomes annosus, in the laboratory, 63; preservation of stone fruit viruses, 628; transference of strawberry virus complex by excised-leaf grafts, 371
Mexico, 396, 411
Michigan, 114, 838
Microstroma juglandis, associated with brooming and galling of shagbark hickory, 448
Mineral oil, for storage of Helminthosporium spp., 290; longevity of certain fungi preserved under, 192
Minnesota, 128, 360, 772, 813
Mississippi, 8, 55, 122, 617, 677, 682
Missouri, 521
Momordica balsamina: wild reservoir for watermelon mosaic virus in Hawaii, 409
Monilinia fructicola, 31, 221, 476, 861
Mycosphaerella citrullina, 883
--- melonis, 41
--- musicola, 758
Myrothecium spp., seed-borne in small grains, 1st rept., 111
Myzus persicae, 240, 409, 565

Necrosis, stem and crown, of Coastal

(Necrosis) Bermuda grass, 498
Needle blight, of Arizona cypress, symptoms and control, 364
Needle cast, associated with Cladosporium spp., on larch and spruce in U.S. (Maine), 265
Nematocide and fungicide tests, results in 1961, 294
Nematocides (see also antibiotics, fungicides, herbicides, insecticides, etc.)
---: Cynem, for control of sting nematode on peanuts, 640
---: Cynem and DBCP, for control of nematodes in outdoor rose plantings, not practical, 610
---: Cynem dip, for control of root-knot nematode in sweetpotato, 21
---: D-D, for control of nematodes of tobacco in India, 173
---: DBCP, effect on nematodes in established grape vineyards, 516
---: effect on root knot, seedling disease complex of cotton and plant growth, 501
---: for root knot on Caryopteris, 186
---: method for assaying, 742
---: methyl bromide, for control of Pratylenchus penetrans on white clover, 346
Nematode-predaceous fungi, collected in North Carolina, 872
Nematodes (see also genera, hosts, nematocides, etc.)
Nematodes, associated with grape in established vineyards, effects of DBCP, 516; holly in British Columbia, 92, in North Carolina, control, 328; little-leaf affected and healthy shortleaf pines, 710; outdoor rose plantings, control with Cynem and DBCP not practical, 610; poor growth of apple trees in West Virginia, 281; stunted tobacco in India, D-D for control, 173; wild yam in Guatemala, 858
---: interrelationship with fungus diseases in Peru, 171
---: method for determining mortality of, 383
---: root observation box for studying, 199
---: widespread in strawberry fields in Nova Scotia, 586
Nematospora coryli, 220
New diseases: Botrytis inflorescence blight of holly, in New Jersey, 320
---: chlorotic fleck (undescribed virus) of strawberry, in Louisiana, 762
---: eyespot (undescribed foliar disease) of banana, caused by Drechslera gigantea, in Jamaica, 305
---: Fusarium wilt of safflower, in California, 748
---: mosaic virus of Kalanchoë flammea, in Florida, 692
---: tatter leaf (undescribed virus), effect on citrus in California, 211
---: tobacco ringspot virus of American spearmint, in Indiana, 623
---: undescribed plant diseases recorded in Oregon 1960-61, 30
---: weak peach, new virus of peach in Georgia, 287
---: wilt of corn in Egypt, 481
---: zonate ringspot virus on Clerodendrum thomsoniae, in Florida, 279
New distribution: bacterial leaf spot of poinsettia in U.S. (Florida), 551
---: Cladosporium spp. associated with needle cast of larch and spruce in U.S. (Maine), 265
---: crazy top (Sclerospora) of corn in Mississippi, 8
---: diseases not previously reported in Oregon, 30
---: Ditylenchus dipsaci on alfalfa in Peru, 171
---: Dolichodorus (undescribed species) in Peru, 171
---: Dutch elm disease in Oklahoma, 463
---: Elsinoë and Sphaceloma spp., 726
---: Fusarium solani f. phaseoli on bean in Hawaii, 819
---: Heterodera humuli on hops in U.S. (Washington), 541, on hops in Oregon, 702
---: kernel-spot of filberts in U.S. (Oregon), 220
---: Phytophthora parasitica var. macrospora on custard apples in India, 874
---: powdery mildew on climbing strawberry in Florida, 348
---: Radopholus similis on banana in Peru, 171
---: Ramularia schulzeri in New York, 655
---: Rhynchosporium leaf scald of rice, in El Salvador, 293, in Guatemala, 202
---: Rotylenchulus reniformis on banana, cocoa, cotton, orange and tobacco plants in Peru, 171
---: rust of medlar in U.S. (Ind.), 293; of peanut in North Carolina, 65, in Virginia, 65
---: Sphaerotheca macularis, perfect stage, on strawberry in North America (Canada), 218
---: Stemphylium leaf spot of cotton in Louisiana, 744
---: target blotch of sugarcane in U.S.

(New distribution) (Fla.), 486
---: Tuberculina maxima on white pine in Michigan, 750
---: white tip disease of rice, in El Salvador, 293
New Hampshire, 130, 346, 379, 839
New hosts: Belonolaimus longicaudatus on grape (Florida), 446
---: Cladosporium sp. on Picea glauca, in Maine, 265
---: Elsinoë and Sphaceloma spp., 726
---: fig mosaic virus on additional Ficus spp., 693
---: Helminthosporium spiciferum on Coastal Bermuda grass in Alabama, 498; Helminthosporium spp. from corn on Gramineae, 321
---: Panicum mosaic virus on Panicum decompositum, P. ramosum, P. turgidum and Setaria verticillata, 780
---: Phytophthora palmivora on Achras sapota in India, 381
---: Ramularia schulzeri on birdsfoot trefoil in New York, 655
---: recorded in Oregon, 30
---: Rhizoctonia sp. on Corsican sandwort in Mass., 172; on cypress euphorbia in Mass., 172; on snow-in-summer in Mass., 172
---: Rotylenchulus reniformis in Louisiana, 683
---: Sclerotium rolfsii in Israel, 418
---: tobacco ringspot on 13 weed species in Texas, 5
New Jersey, 140, 190, 277, 320, 393, 513
New York, 24, 111, 285, 404, 583, 601, 631, 655
New Zealand, 570
Nigeria, 38
North Carolina, 65, 328, 438, 640, 710, 766, 872
Northern leaf blight, of corn in Pennsylvania, 657; winter survival studies in Wisconsin, 444
Nova Scotia, 586
Nyssa sylvatica var. biflora: Fusarium solani in Louisiana, 732

Oak anthracnose, in Mississippi, 677
Oats: Ascochyta leaf spot, in Kentucky, 818
---: barley yellow dwarf virus, varieties tested in field and greenhouse for resistance, 579
---: Glomerella cingulata, seed borne, 1st rept., 111
---: Myrothecium roridum, Myrothecium verrucaria, seed borne, 1st rept., 111
---: Puccinia coronata avenae, new races reduced forage yields in Texas, 835
---: Puccinia coronata avenae, race 264, resistance not induced by seed irradiation, 88
---: Puccinia graminis avenae in Colombia, physiologic races and subraces, 869
---: soil-borne mosaic, winter varietal reactions, 438
Ohio, 124, 186, 401, 560
Oil sprays, for control of Cercospora diseases, inadequate results, 105
Oklahoma, 236, 463
Olea europaea: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
Olpidium brassicae, 428
Oncometopia undata, 292
Onion: Pratylenchus penetrans, seedling injury associated with population density, 484, susceptibility of varieties, wild onion source of resistance, 535; Trichodorus christiei, pathogenic to, 24
Orange: Rotylenchulus reniformis, 1st rept. from South America (Peru), 171
Orchidaceae: Phytophthora palmivora, host range studies and the role of temperature in disease development, 643
Oregon, 30, 220, 555, 699, 702
Ozone (see also Air pollutants), methods and equipment for measuring low concentrations, 137

Pacific Northwest, 784
Panicum decompositum: Panicum mosaic virus, 1st rept. on this host (Kansas), 780
--- maximum: chlorosis due to iron deficiency in Nigeria, 38
--- --- var. gramalote: Cercospora fusimaculans, oil sprays for control (inadequate results), 105
--- ramosum: Panicum mosaic virus, 1st rept. on this host (Kansas), 780
--- turgidum: Panicum mosaic virus, 1st rept. on this host (Kansas), 780
Parsley: Fusarium wilt, 1st rept. in Oregon, 30
Paspalum notatum: Sclerotinia blight, in Mississippi, 55
Peach: ammonia injury in storage, 792
---: bacterial spot, control with dodine-sulfur, 251
---: brown rot, control with fungicides-additives, 476
---: Coryneum blight, control with organic fungicides, in California, 688
---: gummosis, caused by Valsa leucostoma, variation in fungus virulence, 274

(Peach) Meloidogyne javanica, susceptibility of rootstocks, in Israel, 531
---: postharvest decays, control, 31, reduced by hot-water treatments, 861, hydrocooling with chemical solutions and chemically treated ice, 221
---: scab, control with fungicides-additives, 476
---: sudden decline in Georgia, physical factors probably responsible, 831
---: tomato ringspot and peach yellow bud mosaic viruses, serologically indistinguishable, 770
---: weak peach, a new virus disease, in Georgia, 287
Peanut: Aspergillus crown rot, in Georgia, 888
---: Cercospora personata, oil sprays for control (inadequate results), 105
---: rust disease, 1st rept. in North Carolina, 65, 1st rept. in Virginia, 65
---: sting nematode, Cynem for control, effect on peanut yield, 640
Pear: decline syndrome, induced by pear psylla, 59
---: disease symptoms in Greece, similar to pear decline and moria, 150
---: fire blight, control with antibiotic sprays, 397
---: viruses (ring spot and stony pit), mechanically transmitted to herbaceous hosts, 338
Pear psylla, associated with pear decline, 59
Pecan: delayed foliation and dieback (cause undet.), in Georgia, 894; scab, control in Alabama, increased returns in quality and production 232, in Oklahoma, 236
Pelargonium sp.: Verticillium albo-atrum, diverse symptoms, 349
Pellicularia filamentosa (see also Rhizoctonia solani), perfect stage, on fescue and clover, in Georgia, 661
--- rolfsii, 41
Penicillium digitatum, 100; effect of biphenyl on germ tube development and control of citrus green mold among isolates of, 115
--- funiculosum, 312
--- sp., 342
--- vermoeseni, causing death of Mexican palm trees in California, 750
Pennsylvania, 163, 194, 657, 729
Pepper: bacterial and Cercospora leaf spots in Florida, soil treatment and spray drenches for control, 652
---: bacterial spot, streptomycin-resistant and susceptible strains, 389
---: Phytophthora blight in Florida, soil treatment and spray drenches for control, 652
---: potato Y virus, mutant strain from graft-inoculated scion YRP10, 569
---: Pratylenchus penetrans, pathogenicity influenced by host nutrition, 472
---: tobacco-etch virus in Texas, host range studies, 565
---, sweet: bacterial spot, 1st rept. on this host in Israel, 175
Peronospora parasitica, 310
--- viciae, unusual occurrence in Pacific Northwest in 1962, 784
Peru, 171
Pesticide drenches, for control of soil-borne pests in celery seedbeds, 441
Petunia hybrida: Phytophthora crown rot in Colorado, control, 506
Philaenus leucophthalmus, associated with Helminthosporium spiciferum on Coastal Bermuda grass, 498
Philippines, 686
Phloxine B (C.I. No. 778), differentiates living from non-living nematodes in plant tissue after hot-water treatments, 383
Phoma sp., of Vinca major, in Oregon, 30
Phomopsis juniperovora, 364
--- sp., on Cunninghamia (new host), 30; Mahonia aquifolium (new host), 30; Rhododendron in Oregon, 30; Torreya (new host), 30
Physalis floridana: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Physiological studies, host-nutrition-nematode pathogenicity relationships, 472
Phytophthora blight, of pepper in Florida, 652; potato in Israel, 145
--- capsici, 41, 652
--- cinnamomi, 570
--- infestans, 130, 145
--- palmivora, of Achras sapota, 1st report on this host (India), persists through soil, "mummies" and seed, 381; of Orchidaceae, pathogenicity studies, role of temperature, 643
--- parasitica, on petunia in Colorado, control, 506
--- --- var. macrospora, on custard apples, 1st rept. in India, 874
--- --- var. nicotianae, 825
--- phaseoli, 393
--- --- strain "B", 617
--- rot, of watermelon, 41
--- spp., effect of oxygen concentration

(Phytophthora) on growth, 606
Picea abies: Cladosporium spp., associated with needle cast, 1st rept. in N. A. (Maine), 265
--- glauca: Cladosporium spp., associated with needle cast, 1st rept. on this host, (Maine), 265
--- spp.: Cytospora canker in Wisconsin, associated with heavy nematode population, 17
Pieris japonica: twig blight, 1st rept. on this host (Oregon), 30
Pinus echinata: Fomes annosus, isolation methods, 674, lab method of obtaining sporophores, 63, widely distributed in southern Missouri, 521
--- ---: littleleaf-like disease, in New Zealand, 570
--- ---: nematodes associated with littleleaf complex, 710
--- elliottii: Fomes annosus, and associated fungi in stumps, in south Georgia, 738, in South Carolina, 64
--- ponderosa: chlorotic decline in southern Calif., drought or air pollution possible causes, 269
--- spp.: Fomes annosus, causing death of trees in California, 107; Tuberculina maxima, 1st rept. in Michigan, 750
--- strobus: basal canker disease, in New York, 285; blister rust, antibiotics-oil carrier for control, 306; Fomes annosus, spore dispersal and invasion, associated with thinning and weather conditions, 194, widespread problem in thinned natural stands in West Virginia, 734
--- taeda: Fomes annosus, stump protectant against, 340
Piricularia grisea, 354
Pisum sativum: Aphanomyces euteiches, oospore method of evaluating varietal resistance or susceptibility, 777; Aphanomyces root rot, control with amino compounds, 646; downy mildew, unusual occurrence in Pacific Northwest in 1962, 784
Pitting disease, of Central American bananas on the market, 354
Poa pratensis: powdery mildew, reduction in growth caused by, 724
Polyporus spp.: interfertility studies, 26
Polystichum munitum: Aphelenchoides fragariae, discussion of its discovery, distribution and symptomatology, 699
Poncirus trifoliata x Citrus paradisi: rootstock incompatibility with Eureka lemon scions, 267
Populus tremuloides: Cenangium singulare, causal agent of sooty-bark canker of aspen, 57
Portulaca oleracea: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Postharvest decays, of peach, control, 31; reduced by hot-water treatments, 861, postharvest fungicides, 221
Potato: Bacillus polymyxa on decayed tubers, in Israel, 893
---: curly top, current-season and tuber-perpetuated symptoms, in Utah, 176
---: Phytophthora blight, effect of irrigation methods, in Israel, 145
---: russet scab, Streptomyces sp. causal agent in Minnesota, 128
---: scab and Rhizoctonia, control with new soil fungicides, 601
---: soil treatments, effect on yields and flavor, 631
Pouteria campechiana: Elsinoë lepagei, 1st rept. in Mexico, 726
Powdery mildew, on barley, effect of liming of soil, 886
--- ---: on climbing strawberries in Florida, (1st rept.), 348
--- ---: on Kentucky bluegrass, reduction in growth, 724
--- ---: on safflower in California greenhouse, 203
--- ---: on strawberry in Louisiana, 591
Pratylenchus alleni, injury to soybeans in Illinois, 181
--- penetrans, effects of paper and sawdust soil amendments, 745; nonpathogenic to low-bush blueberry, 84; on onion, 484; on pepper, pathogenicity influenced by host nutrition, 472; on strawberry, role in black root rot complex, 839; on white clover in New Hampshire, 346; susceptibility-resistance of onion varieties, 535
--- spp., associated with economic crops in Nile Delta Valley, 572; widespread in strawberry fields of Nova Scotia, 586
--- vulnus, pathogenic to boxwood, 712
Preservation, of fungi under oil, 192, 290
Prunus (see also under cherry, peach, etc.)
--- amygdalus: air-carrier spraying, effect of volume and droplet size on disease control, 614
--- domestica: Cytospora sp., factors affecting invasion, 45, 77
--- spp: bud-perpetuated conditions, interrelationships of, and variations with time, 523; crinkle leaf, stability of symptoms, 528; peach mosaic virus, varietal reactions, 495

Pseudomonas andropogonis, 98
--- angulata, 36
--- caryophylli, effect of plant growth regulators, 469
--- sesami, second pathogenic race of, 248
--- sp., on African violet in Oregon, 30; gloxinia in Oregon, 30
--- tabaci, 36
Puccinia andropogonis, life cycle and alternate host relationship, 706
--- arachidis, 65
--- coronata avenae, 88, 835
--- graminis avenae, of oats in Colombia, new races, 869
--- --- tritici: effect of thawing temperature on urediospores frozen in liquid nitrogen, 299
--- phragmitis, life cycle and alternate host relationships, 706
--- sorghi, 14
--- sporoboli var. robusta, life cycle and alternate host relationships, 706
Puerto Rico, 758
Pullularia pullulans, 342
Punica granatum: Sphaceloma punicae, 1st rept. in Dominican Republic and Texas, 726
Pythium sp(p.), 122, 539

Quercus spp.: anthracnose, symptoms and etiology, in Mississippi, 677; leaf-blister, heavy local infection in Mississippi, 682

Races (see also strains): Pseudomonas sesami, new pathogenic race, 248
---: Puccinia coronata avenae, new races in Texas, 835; race 264, seed irradiation studies, 88
---: Puccinia graminis avenae in Colombia, new, 869
---: Uromyces phaseoli typica, 15 new races in Mexico, 411
---: Xanthomonas malvacearum, distribution in Arkansas, 168
Radish: yellows, seasonal variation and effect of crop rotation, in Ohio, 124
Radopholus similis, on banana, 1st rept. in Peru, 171; on citrus seedlings, chemicals tested for therapeutic action against, 430; on grapefruit seedlings, movement from infected to non-infected potted plants, 735
Ramularia schulzeri, on birdsfoot trefoil, 1st rept. on this host and in New York, 655
Raphiolepis indica: leaf spot, 1st rept. on this host (Oregon), 30
Reaction (see also resistance, susceptibility, etc.): of beans to strains of tobacco mosaic virus, 537
---: of plum varieties to peach mosaic virus, commercial rootstocks versus own-roots, 495
---: of soybeans to Xanthomonas phaseoli var. sojensis, 707
---: of winter oats to soil-borne mosaic, 438
Resistance (see also reaction, susceptibility): of alfalfa breeding lines to Meloidogyne spp., 558
---: of Allium canadense to Pratylenchus penetrans, 535
---: of corn inbred lines to bacterial wilt and leaf blight, 277
---: of oat varieties to barley yellow dwarf virus, 579
---: of Orchidaceae to Phytophthora palmivora, 643
---: of tomato variety Rockingham to late blight, 130
---: of watermelon introductions to gummy stem blight, 883
Rhizobium japonicum, studies on soybean nodulation in cyst-nematode-infested soil, 48
Rhizoctonia blight, of tall fescue grass in Georgia, 661
--- root rot, of snap beans in Florida, control, 651
--- solani (see also Pellicularia filamentosa), 122, 227, 258, 312, 501, 601, 651, 661, 852; lytic effects of Streptomyces sp. on, use of nylon mesh in studies, 616; on bean seed in Israel, 790
--- sp., on snow-in-summer, cypress euphorbia, and Corsican sandwort in Mass., 1st rept. on these hosts, 172
Rhizopus nigricans, 31
--- rot, of peaches, control, 31, 221, 861
--- stolonifer, 221, 861
Rhododendron: twig blight and stem canker, 1st rept. in Oregon, 30
Rhynchosporium oryzae, 202, 293
Rice: Rhynchosporium leaf scald, 1st rept. in El Salvador, 293, 1st rept. in Guatemala, 202; white tip disease, 1st rept. in El Salvador, 293
Root rot, in thinned white pine stands in West Virginia, 734; of strawberry, control, 258
Rosa spp.: nematodes, Cynem and DBCP for control not practical, 610; Prunus ringspot virus, determined in rose virus isolates, 722; rose mosaic and tomato ring spot, closely related, 555
Rotylenchulus reniformis, new hosts and non-hosts, in Louisiana, 683;

(Rotylenchulus) on various hosts, 1st rept. from South America (Peru), 171
Russet scab, of potato, 128
Rust, of peanut, 1st rept. in North Carolina, 65, 1st rept. in Virginia, 65
Rusts (North American), life cycles and alternate host relationships of three rust fungi occurring in Great Lakes region, upper Midwest and the Plain States, 706

Safflower: see Carthamus
Sagina subulata: Rhizoctonia sp., 1st rept. on this host (Mass.), 172
Salix nigra: Sphaceloma murrayae, 1st rept. on this host and in Texas, 726
Scab, of almond, control, 614; apple, control in Delaware, 159, in Pennsylvania, 163; cucumber, inhibited by mosaic, 93; peach, control, 476; pecan, control, 232, 236; potato, control, 601
Scald, of apple, control, 808
Sclerospora macrospora, 8
Sclerotinia blight, of pasture grasses in Mississippi, 55
--- homeocarpa, 55
--- rolfsii, 492, 651; on new hosts in Israel, 418
--- rot, of watermelon, 41
Secondary organisms, associated with blossom-end rot of watermelon, 41; kernel-spot of filberts, 220; post-harvest deterioration of downy mildew-susceptible lettuce, 719; Sclerotinia blight of pasture grasses, 55
Seed-borne diseases: see Transmission
Seed irradiation, X-ray, in attempt to induce crown rust resistance in oats, 88
Seed sterilization: wheat, by ethylene oxide, 540
Seedling disease complex, of cotton, fungi involved, 122; influence of root-knot nematodes, 501
Seedling losses, of alfalfa, relationship of pre-emergence and postemergence, 597
Selenophoma donacis, 728
--- spot, on 'Gaines' wheat in Washington, 728
Septoria tritici, of wheat, effects of light, temperature and fertilizer, 846
Sesamum indicum: new pathogenic race of Pseudomonas sesami, 248
Setaria verticillata: Panicum mosaic virus, 1st rept. on this host (Kansas), 780
Sigatoka leaf-spot, of banana in Puerto Rico, effect of climatic factors, 758
Soil amendments: effect of paper and sawdust on Pratylenchus penetrans and Verticillium wilt of tomatoes, 745; effect of organic materials on citrus nematode, 375
Soil fumigation (see also soil treatment, nematocides, etc.): effect on nodulation of soybeans in soybean-cyst-infested soil, 48; for control of Agrobacterium tumefaciens, 73; for control of nematodes and weeds in holly nurseries, economic gains, 328
Soil treatments (see also fungicides, nematocides, soil fumigation, etc.): effect on yields and flavor of carrots and potatoes, 631; for control of Fusarium wilt of tomato, 151; with DAC 699 and Hercules 3944 for control of Rhizoctonia root rot and southern blight of snap beans in Florida, 651; with fungicides with and without herbicides, for control of tomato fruit diseases, 227; with sodium hypochlorite, not effective against Dutch elm disease, 216
Soil type, possible cause of basal canker disease of eastern white pine, 285
Soil-borne pests, in celery seedbeds, control studies, 441
Solanum diphyllum: tobacco ringspot virus, 1st rept. on this host (Texas), 5
Sooty-bark canker, of aspen in Colorado, 57
Sooty blotch, of apple, control, 513
Sore-shin, of cotton seedlings, greenhouse technique for evaluating fungicides for control, 852
Sour rot, of citrus in Louisiana, 185
South Africa, 487
South Carolina, 64, 438, 492, 710
Southern blight, of snap beans in Florida, control, 651; of tomato, chemical control, 492
Southern leaf blight, of corn hybrids in Pennsylvania, 657
Soybean: effects of seed inoculation, soil fumigation, and cropping sequences on nodulation in soybean-cyst-nematode-infested soil, 48
---: Heterodera glycines, physiological strains, 766
---: Pratylenchus alleni, reduction in root growth, 181
---: Xanthomonas phaseoli var. sojensis,

(Soybean) reaction of resistant and susceptible varieties to, 707
Sphaceloma murrayae, on Salix nigra, 1st rept. on this host (Texas), 726
--- poinsettiae, on Euphorbia pulcherrima, 1st rept. in Arizona and Texas, 726
--- punicae, on Punica granatum, 1st rept. in Dominican Republic and Texas, 726
--- spondiadis, on Spondias cytherea "vi-apple", 1st rept. in British Guiana and Puerto Rico, 726; on Spondias lutea "jubo", 1st rept. on this host, (Barbados), 726
--- zorniae, on Zornia reticulata, 1st rept. on this host (St. Kitts, Leeward Islands), 726
Sphaerotheca humuli, 591
--- macularis, 348; the perfect stage on strawberry, 1st occurrence in N. A. (Canada), 218
Spinach: mottle and distortion, 1st rept. in Oregon, 30
Spondias cytherea "vi-apple": Sphaceloma spondiadis, 1st rept. in British Guiana and Puerto Rico, 726
--- lutea "jobo": Sphaceloma spondiadis, 1st rept. on this host and in Barbados, 726
Stagonospora bromi, on Bromus inermis, 729
Stalk rot, of corn, 9, 18; of corn hybrids in Pennsylvania, effect of Helminthosporium on severity of, 657
Stayman spot, of apple, control, 808
Stem-end rot, of watermelon, 41
Stemphylium leaf spot, of birdsfoot trefoil, control, 509; of cotton, 1st rept. in Louisiana, 744
--- loti, 509; on blue lupine breeding lines, 333
Stenotaphrum secundatum: effects of sting and stubby-root nematodes, 424; Meloidogyne sp., severe infestation in Florida, 52
Strains (see also races): citrus psorosis virus, infection variegation strain, mechanically transmitted to cucumber, 414; Heterodera glycines on soybeans, 766; lima bean downy mildew, strain "B", spread in New Jersey - 1960, 617; Penicillium digitatum, differential sensitivity to sodium orthophenylphenate, 100; potato Y virus from pepper, 569; Pseudomonas spp. on resistant tobacco varieties, 36; tobacco mosaic virus, reaction of bean to, 537; Xanthomonas vesicatoria, streptomycin-resistant and -susceptible, 389
Strawberry (see also Fragaria): anthracnose and powdery mildew in Louisiana, 591
---: bacterial angular leafspot, epidemiology studies, 360
---: gray mold rot, biological control studies, 342
---: necrotic shock (graft-transmissible virus), in California, 547
---: nematodes, found in a field survey in Nova Scotia, 586
---: peach yellow bud mosaic virus, transmitted by Xiphinema americanum, 303
---: Pratylenchus penetrans, role in black root rot complex, 839
---: Rhizoctonia solani infestation, effect of fungicidal drenches, 258
---: Sphaerotheca macularis, perfect stage, 1st occurrence in N. A. (Canada), 218
---: two-spotted spider mites, effect of fungicidal drenches, 258
---: Verticillium wilt, influence of time of planting and runner removal, 190
---: virus complex inoculation by grafting, varietal tolerance determination, 371
---: virus diseases in Louisiana, effect on plant production and fruit yield, 762
Streptomyces scabies, 601
--- sp., causal agent of potato russet scab, 128; lytic effects on Rhizoctonia solani, use of nylon mesh in studies of, 616
Sucrose, Heterodera schachtii cysts pretreated with, effect on emergence of larvae, 43
Sugarcane: mosaic virus, inhibited by milk, 213; target blotch, 1st rept. in U.S. (Fla.), 486
Sulfur dioxide, see Air pollutants
Survey, of plant diseases in Kentucky in 1961, 372
Susceptibility (see also reaction, resistance): of blue lupine breeding lines to Stemphylium loti, 333
---: of lettuce to downy mildew, factor in postharvest deterioration, 719
---: of onion varieties to Pratylenchus penetrans, 535
---: of Orchidaceae to Phytophthora palmivora, 643
---: of peach rootstocks to root-knot nematodes, 531
---: of wildfire-resistant tobacco varieties to strains of Pseudomonas spp., 36
Sweden, 152
Sweetpotato: black rot, sprout dip treat-

(Sweetpotato) ments for control and effect on yields, 253; chloropicrin injury, 246; feathery mottle in var. Georgia Red, mixture of three virus diseases, 796; internal cork virus, heat treatment for, (negative results), 19; Meloidogyne incognita acrita, control with Cynem dip and hot-air treatments, 21
Swietenia sp. : Dolichodorus (undescribed species) in soil, 1st rept. of this genus from South America (Peru), 171

Taphrina coerulescens, 682
Target blotch, of sugarcane, 1st rept. in U.S. (Fla.), 486
Taylor, Dr. Thomas, and cotton seed delinting, 230
Techniques and apparatus (see also method): agitator-incubator for aseptic culturing of virus-infected cells and tissue in liquid media, 456
---: air-carrier sprayer operating techniques and application equipment for control of almond diseases, 614
---: determining oxygen requirement of fungi in liquid media, 606
---: greenhouse screening of fungicides for control of cotton sore-shin, 852
---: heat chamber for curing virus diseased plants, 703
---: inexpensive temperature tanks, description of, 61
---: measuring low concentrations of ozone, 137
---: microvolumeter for measuring the volume of plant tissue cultures, 459
---: root observation box for studying infection and pathogenesis of roots by nematodes, 199
---: soil fungi studies using nylon mesh, 616
Temperature, effect on Septoria infection of wheat leaves, 846; effect of thawing on urediospores of Puccinia graminis tritici frozen in liquid nitrogen, 299; factors in control of black spot of citrus in South Africa, 487; role in development of Phytophthora palmivora in the orchid genera, 643
Tennessee, 48, 766
Tetranychus telarius, 258
Texas, 5, 151, 200, 565, 835, 882
Thielaviopsis basicola, 501; effect of oxygen concentration on growth, 606; prevalent in citrus soils in California, 357
--- sp., on fuchsia in Oregon, 30
Thuja: tip blight, 1st rept. on this host (Oregon), 30
Tilletia barclayana, 64
Tobacco: black shank, lab method of detecting disease potential index in infested soil, 825; Cercospora nicotianae, oil sprays for control (negative results), 105; frogeye in Venezuela, control by isolation of plant beds from native vegetation, 461; mosaic virus, effect of CCC on multiplication of, 170; nematodes in India, control, 173; Pythium sp., in plant beds in Kentucky, 539; Rotylenchulus reniformis, 1st rept. from South America (Peru), 171; wildfire-resistant varieties, susceptibility to strains of Pseudomonas spp., 36
Tomato: bacterial spot, in Israel, 175; streptomycin-resistant and susceptible strains, 389
---: fruit diseases, control with soil treatments, 227
---: Fusarium wilt in Texas, control with Vorlex, 151
---: ghost spot, unusual epidemic in Maryland, 260
---: late blight, new resistant variety, 130
---: leaf blight, 1st rept. in Oregon, 30
---: Pratylenchus penetrans and subsequent Verticillium wilt, effects of paper and sawdust soil amendments, 745
---: southern blight in South Carolina, calcium nitrate as a side-dressing for control, 492
---: tobacco-etch virus in Texas, host range stduies, 565
---: Verticillium wilt, following nematodes, effect of paper and sawdust soil amendments, 745
Torreya: necrotic leaf spot, 1st rept. on this host (Oregon), 30
Transmission, of bean mosaics through seed, 71; of gladiolus viruses on tools in harvesting of flowers and corms, 505; of Glomerella cingulata, by seeds of small grains, 111; of Myrothecium spp., by seeds of small grains, 111; of peach yellow bud mosaic virus by Xiphinema americanum, 303; of Pierce's disease (virus) of grape by leafhopper vectors, 292; of strawberry virus complex by grafting, 371
--- contact, of viruses, role of trichomes, 317
Treatments, for control of Dutch elm disease, negative results, 401; for eradication of Ditylenchus dipsaci in cloves of garlic, 635; of stumps against Fomes annosus, 340
Trees: diseases in Japan, 34; Polyporus spp., genetic studies, 26; urethane foam for filling cavities, 384

Trichoderma viride, on lemon, spread by contact infection in nonvented versus vented cartons, 804
Trichodorus christiei, effect on St. Augustine grass, 424; pathogenic to onion, 24
Trichothecium roseum, 379
Trifolium alexandrinum: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
--- repens: nematodes associated with, in New Hampshire, 346; Pellicularia filamentosa on Ladino var., in Georgia, 661
Tritonia sp.: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
Tuberculina maxima, on white pine, 1st rept. in Michigan, 750
Tylenchulus semipenetrans, occurrence and distribution in Florida, 451; reduction of soil populations by addition of organic materials, 375

Ulmus americana: Dutch elm disease, control studies, negative results, 401, 1st rept. in Oklahoma, 463, root graft unions, 86, sodium hypochlorite for control not effective, 216, spread in Kansas, 189
United Arab Republic, 572
Urea, stump protectant against Fomes annosus, 340
Urethane foam, possible use for filling cavities in trees, 384
Uromyces caryophyllinus, 152
--- phaseoli typica, 15 new races in Mexico, 411
Utah, 176, 665

Vaccinium spp.: Helicotylenchus sp., distribution in the Maritime Provinces, 800
Valeriana italica: Sclerotium rolfsii, 1st rept. on this host (Israel), 418
Valsa leucostoma, variation in virulence on peach varieties, 274
Vectors, aphid, of bean yellow mosaic virus, control, 240; of tobacco-etch virus, 565
--- leaf hopper, of Pierce's disease virus of grape, in Georgia, 292; of virus diseases in Michigan, 114
---, nematode, of peach yellow bud mosaic virus of strawberry, 303
Venezuela, 461
Venturia inaequalis, 159, 163
Verticillium albo-atrum, 190, 665, 745; on geraniums, diverse symptoms, 349
--- wilt, of safflower, 665; of strawberry in New Jersey, 190; of tomatoes, severity reduced by control of meadow nematodes with soil amendments, 745
Vinca major: stem canker, 1st rept. in Oregon, 30
Virginia, 65, 340, 509, 710
Virus, Virus disease:
---: of apple (stem pit, dapple apple, flat limb and mosaic), comparative symptoms, 80; of cantaloup in Arizona, distribution, prevalence and role in the crown blight complex, 667; of gladiolus, transmitted on tools in harvesting flowers and corms, 505
---: aster yellows in flax, effect of systemic insecticides, 813; aster yellows, mottle and vein banding viruses of strawberry, effect on plant production and fruit yield in Louisiana, 762
---: banana mosaic in Honduras, 366
---: barley yellow dwarf of oats, varietal reactions, 579
---: bean mosaics, role of seed transmission, 71
---: bean yellow mosaic of yellow lupine, indirect control with systemic insecticides, 240
---: big vein of lettuce, 428
---: cadang-cadang of coconut, intracellular inclusion bodies observed, 686
---: chlorotic fleck (undescribed virus) of strawberry, in Louisiana, 762
---: citrus psorosis infectious variegation strain, mechanically transmitted to cucumber, 414
---: contact transmission of viruses, role of trichomes, 317
---: cowpea mosaic viruses, isolated from gladiolus, 335
---: cucumber mosaic, eliminated from Easter lilies by scale propagation, 627; inhibitory effect on development of cucumber scab, 93; of safflower, in California, 849; of spinach, in Oregon, 30
---: curly top of potato, current-season and tuber-perpetuated symptoms, 176
---: feathery mottle in Georgia Red sweetpotato, mixture of three virus diseases, 796
---: fig mosaic, additional Ficus hosts, 693
---: green ring mottle, not seed-transmitted to mazzard cherry seedlings, 356
---: heat chamber for curing virus diseased plants, 703
---: internal cork of sweetpotato, 19
---: latent A virus of Fragaria vesca,

(Virus) method of excluding, 119; of strawberry, F. virginiana an effective indicator host, 772
---: latent viruses of apple, Hopa crab effective index host, 243
---: leafhopper vectors of, in Michigan, 114; of Pierce's disease, in Georgia, 292
---: lily ringspot virus from Georgia Easter lilies, 625
---: mosaic of Kalanchoë flammea in Florida, 1st rept. of a virus in this genus, 692
---: necrotic shock of strawberry, endemic in California, 547
---: necrotic ring spot and sour cherry yellows, in commercial mazzard and mahaleb cherry rootstocks, 583
---: Panicum mosaic on new hosts, 780
---: peach mosaic on plum, varietal reactions, 495
---: peach yellow bud mosaic of strawberry, transmitted by Xiphinema americanum, 303
---: pear decline in Greece possibly due to virus, 150
---: Pierce's disease on grape, transmitted by leafhopper vectors, in Georgia, 292
---: potato Y virus, mutant strain from pepper, 569
---: Prunus ringspot virus, presence in rose virus isolates, 722
---: ring mosaic and stony pit of pear, mechanical transmission to herbaceous hosts, 338
---: rose mosaic, possible strains of tomato ring spot, 555
---: soil-borne mosaic of oats, winter varietal reactions, 438
---: sour cherry yellows influence of gibberellin sprays on infected trees, 404
---: stone fruit viruses, method of preservation, 628
---: strawberry virus complex transmission by grafting as means of testing varietal tolerance, 371
---: sugarcane mosaic virus, inhibited by milk, 213
---: "tatter leaf" (undescribed virus) on Citrus spp., in California, 211
---: tobacco etch on bell pepper and tomato in Texas, 565
---: tobacco mosaic, bean reaction to tomato and tobacco strains of, 537; inhibitory effect of the growth regulator CCC on multiplication of virus, 170
---: tobacco ring spot, cause of new disease of American spearmint, 623; on papaya, in Texas, 882; on weed species (13 new hosts) in Texas, 5
---: tobacco ringspot, and tomato ringspot viruses, differential symptom reactions on various hosts, 877
---: tomato ring spot, of Iris in Oregon, 30
---: tomato ringspot, and peach yellow bud mosaic in peach, serologically indistinguishable, 770
---: watermelon mosaic, host range studies in Hawaii, 409
---: weak peach, new virus of peach, 287; similar symptoms produced in peach by grafts from Prunus angustifolia and Concord grape, 287
---: zonate ringspot, a new disease of bleeding-heart, in Florida, 279

Washington, 59, 71, 579, 722, 728
Washingtonia robusta: Penicillium vermoeseni, causing death in California, 750
Watermelon: see Cucurbits
Weather: possible relation to sudden decline of peach trees in Georgia, 831
Weeds, control, in holly nurseries, 328
West Virginia, 281, 734
Wheat: Glomerella cingulata, seed borne, 1st rept., 111; Myrothecium roridum, Myrothecium verrucaria, seed borne, 1st rept., 111; seed sterilization by ethylene oxide, 540; Selenophoma spot, severe outbreak on "Gaines" variety in Washington, 728; Septoria leaf blotch, effects of light, temperature and fertilizer, 846
White tip disease, of rice, 1st. rept. in El Salvador, 293
Wildfire, of tobacco, 36
Wilt diseases, of carnation in Sweden, 152
Wisconsin, 17, 444
Witches' brooms and galling, of Carya ovata in Arkansas, 448

Xanthomonas malvacearum, 168
--- phaseoli var. sojensis, 707
--- poinsettiaecola, 551
--- pruni, 251; effect of plant growth regulators, 469
--- stewartii, 277
--- vesicatoria, 175, 389, 652
Xiphinema americanum, 710; in soil around canker-diseased spruce in Wisconsin, 17; in strawberry fields of Nova Scotia, 586; on coffee in Guatemala, 857; possible vector of tobacco ringspot virus, causing a

(Xiphinema) new disease of Mentha spicata, 623; vector of peach yellow bud mosaic virus, 303

Yellows, of radish in Ohio, 124

Zinnia: Cercospora zinniae, oil sprays for control (negative results), 105
Zornia reticulata: Sphaceloma zorniae, 1st rept. on this host and in St. Kitts, Leeward Islands, 726

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